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# THE JOURNAL OF AGRICULTURAL SCIENCE

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# Contents

vii

	PAGE
PIZER, N. H. Investigations into the environment and nutrition of the cultivated mushroom <i>Psalliota campestris</i> . I. Some properties of composts in relation to the growth of the mycelium. (With Plate IV) . . . . .	349
BELL, G. D. H. The effect of low-temperature grain pre-treatment on the development, yield and grain of some varieties of wheat and barley . . . . .	377
HAMMOND, JOHN and MURRAY, G. N. The body proportions of different breeds of bacon pigs. (With Plates V, VI and seven text-figures) . . . . .	394
CULPIN, C. Studies on the relation between cultivation implements, soil structure and the crop. III. Rolls: an account of methods employed in a study of their actions on the soil. (With two text-figures) . . . . .	432
GARNER, F. H. and SANDERS, H. G. On the spring cultivation of autumn-sown wheat . . . . .	447
MOON, F. E. and THOMAS, BRYNMOR. The digestion of huskless oats by poultry . . . . .	458
WOODMAN, H. E. and EVANS, R. E. The composition and digestibility, when fed to pigs, of three grades of meat meal of widely differing fat content . . . . .	465
WATSON, D. J. The estimation of leaf area in field crops. (With one text-figure) . . . . .	474

## PART 4 (OCTOBER 1937)

BUCHANAN SMITH, A. D. and DONALD, H. P. Weaning weight of pigs and litter sampling with reference to litter size. (With one text-figure) . . . . .	485
NOWOTNÓWNA, ANNA. An investigation of nitrogen uptake in mixed crops not receiving nitrogenous manure. (With Plate VII) . . . . .	503
HEATH, O. V. S. A study in soil cultivation. The effects of varying soil consolidation on growth and development of rain-grown cotton. (With four text-figures) . . . . .	511

SCOTT BLAIR, G. W. Compressibility curves as a quantitative measure of soil tilth. (With Plates VIII and IX and four text-figures) . . . . .	541
MITCHELL, R. L. Base exchange equilibria in soil profiles . . . . .	557
WOODMAN, H. E., EVANS, R. E. and TURPITT, W. G. The nutrition of the bacon pig. II. The influence of high-protein intake on protein and mineral metabolism . . . . .	569
VIRTANEN, ARTTURI ILMARI, VON HAUSEN, SYNNÖVE and LAINE, TAUNO. Investigations on the root nodule bacteria of leguminous plants. XX. Excretion of nitrogen in associated cultures of legumes and non-legumes. (With Plate X and six text-figures) . . . . .	584
MENZIES, KITCHIN, A. W. Fertility, morality, and growth rate in pigs. (With three graphs) . . . . .	611
VARTIOVAARA, UNTO. Investigations on the root-nodule bacteria of leguminous plants. XXI. The growth of the root-nodule organisms and inoculated peas at low temperatures . . . . .	626
GARNER, F. H. and SANDERS, H. G. The water consumption of suckling sows . . . . .	638

# CONTENTS

## PART 1 (JANUARY 1937)

	PAGE
WATSON, S. J. and FERGUSON, W. S. The chemical composition of grass silage. (With three text-figures) . . .	1
DILLON WESTON, W. A. R., HANLEY, F. and BOOER, J. R. Seed disinfection. II. Large-scale field trials on the disinfection of seed corn with mercury dust disinfectants . . .	43
BRETT, C. C., DILLON WESTON, W. A. R. and BOOER, J. R. Soil disinfection. III. Experiments on the germination of peas. Seed protection by the use of disinfectant dusts containing mercury. (With Plate I) . . .	53
WATSON, S. J. and FERGUSON, W. S. The losses of dry matter and digestible nutrients in low-temperature silage, with and without added molasses or mineral acids . . .	67
MANN, HAROLD H. The character of barley grown on soil made acid with sulphate of ammonia. (With Plate II) . . .	108
BEATER, B. E. Improved technique in grading of coarse and fine sands during mechanical analysis of soils. (With one text-figure) . . .	123
HALNAN, E. T. Digestibility trials with poultry. VII. The digestibility of wheat offals, with a note on the apparent discrepancy between the digestibility coefficients and nutritive values of these products . . .	126
HALNAN, E. T. Digestibility trials with poultry. VIII. The digestibility of dried molassed sugar-beet pulp . . .	137
ANDERSON, JAMES. Artificial insemination of sheep. I. Preliminary investigation and its application to sheep in Kenya . . .	143

## PART 2 (APRIL 1937)

DAVIES, W. MORLEY, BOTHAM, G. H. and THOMPSON, W. B. Grass silage. A comparison of the changes involved in the ordinary, molasses and A.I.V. processes . . .	151
DUTHIE, D. W. Studies in tropical soils. IV. Organic transformations in soils, composts and peat . . .	162



	PAGE
COPEMAN, P. R. v. d. R. and DILLMAN, F. J. Changes in the composition of guano during storage. (With two text-figures) . . . . .	178
✓ HENDRICKS, WALTER A. A note on the method of "differential regression" in analysis of variance . . . . .	188
WOODMAN, H. E., EVANS, R. E. and EDEN, A. Sheep nutrition. I. Measurements of the appetites of sheep on typical winter rations, together with a critical study of the sheep-feeding standards . . . . .	191
WOODMAN, H. E., EVANS, R. E. and EDEN, A. Sheep nutrition. II. Determinations of the amounts of grass consumed by sheep on pasturage of varying quality . . . .	212
WATSON, S. J., FERGUSON, A. S. and HORTON, E. A. The time of cutting hay, and the losses entailed during hay-making . . . . .	224
WILLIAMS, RICE. The solubility of soil phosphorus and other phosphorus compounds in sodium hydroxide solutions . .	259
ALLEN, L. A., HARRISON, J., WATSON, S. J. and FERGUSON, W. S. A study of the chemical and bacteriological changes occurring in grass silage. (With four text-figures) . . .	271
ALLEN, L. A., WATSON, S. J. and FERGUSON, W. S. The effect of the addition of various materials and bacterial cultures to grass silage at the time of making on the subsequent bacterial and chemical changes . . . . .	294
CRUICKSHANK, E. M. A note on the effect of different cereals in the fattening ration on the composition of the body fat of the fowl . . . . .	309

### PART 3 (JULY 1937)

DEIGHTON, THOMAS. A study of the fasting metabolism of various breeds of hog. III. Metabolism and surface area measurements. (With four text-figures) . . . . .	317
VIRTANEN, ARTTURI ILMARI, VON HAUSEN, SYNNOVE and LAINE, TAUNO. Investigations on the root nodule bacteria of leguminous plants. XIX. Influence of various factors on the excretion of nitrogenous compounds from the nodules. (With Plate III and one text-figure) . . .	332

## THE CHEMICAL COMPOSITION OF GRASS SILAGE

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(With Three Text-figures)

## INTRODUCTION

SILAGE is a succulent fodder of high moisture content made from fresh forage crops such as grass, grass-legume mixtures or legumes by storing them in a stack or some suitable container—a silo—in which air is excluded as completely as possible. The changes which take place in the crop may be divided into two classes, those occurring while the cells are still alive and those subsequent to the death of the cell.

In the first category the main losses are due to respiration which continues after the crop is cut. During the filling of the silo with the fresh crop a fairly large volume of air is entangled in the mass, and this gives rise to respiration proper with the evolution of large volumes of carbon dioxide. Even after the exhaustion of this supply, intracellular respiration must take place with the production of alcohol, and certain of the simpler fatty acids, of which acetic is the chief. At the same time, enzyme action is operative, with a resultant simplification of the chemical constituents of the mass, and marked proteolysis may occur. Associated with the changes mentioned above, there is usually a marked increase in the temperature of the mass, and this is inversely proportional to the degree of compaction attained in filling the fresh crop into the container or building it into a stack, since this affects the degree of respiration permitted.

After respiration has ceased and the cells are dead, micro-biological changes set in. This aspect of the problem has been studied by Allen & Harrison<sup>(1)</sup> in collaboration with the authors. To produce the best type of silage it is essential that a rapid lactic fermentation takes place. The acidity thus formed in the mass will prevent the onset of less desirable fermentations such as the butyric acid type. According to Virtanen<sup>(2)</sup>, this depends on the hydrogen-ion concentration of the mass; below pH 4.2 the action is checked.

The control of the silage process depends, therefore, on the com-

paction of the material and exclusion of air to as great a degree as possible, and the rapid acidification of the mass. The former depends entirely on the method of filling and the type of container used.

The success of this part of the operation can be measured by the temperature of the mass. In stacks where compaction, particularly at the edge, is not so easy of achievement, and the mass is open to the air, high temperatures are normally registered. Under the best conditions the temperature at its maximum will be in the neighbourhood of 120° F., but too frequently this is exceeded. This process is spoken of as the *warm fermentation process*, and in a silo is achieved by relatively loose packing. It is obvious that the losses, particularly of carbohydrates, must be high. The so-called *cold fermentation process* aims at keeping the temperature at or below 80° F., and this end is achieved by the chaffing of the material, which is cut into lengths of  $\frac{1}{2}$  in. or so, in order to pack more closely, heavy trampling, and the application of pressure to the silo as dead weight or by mechanical devices. At Jealott's Hill the best results have been obtained by allowing the mass to rise to a temperature of between 80 and 100° F. The silo is filled in layers, each of which is allowed to heat to this temperature, and finally sealed off with a layer of soil and small movable concrete weights. This we call the *low temperature process*.

The aim of modern practice is to obtain a material of as high a protein content as possible. Under these conditions it is not always possible to get a sufficiently rapid formation of lactic acid, due primarily to the relatively lower amount of fermentable carbohydrates present.

Stimulation of lactic fermentation can take two forms: the addition of suitable organisms, or the addition of a fermentable carbohydrate. The former, as might be expected from the fact that the original crop is usually rich in a microflora capable of lactic acid fermentation, has not up to the present proved very successful, though further work is still necessary in this direction.

The addition of fermentable carbohydrates is a useful step. In order to obtain the best results, it is necessary to ensure an even distribution of the medium throughout the mass. Sugar itself has been used, but in this country molasses is the cheapest practical solution of the problem, though whey has also been used with success(3). In the latter case, however, fresh whey is too dilute for the purpose, and it has proved necessary to use a solution of concentrated whey. It would seem that an addition of  $\frac{3}{4}$ –1 per cent of molasses is adequate. Efforts have been made to sterilize the green fodder in the silo, but these have met with no practical success.

The realization that it was the acidity which controls undesirable fermentations led to the addition of acids, organic and mineral, to the fresh fodder, culminating in the *A.I.V. process* put forward by Virtanen (4). In this process a mixture of mineral acids is added in dilute solution in quantities sufficient to bring the mass within a very short time to an acidity of between  $pH$  3.0 and 4.0.

#### EVALUATION OF SILAGE

Apart from practical observations, which are usually devoted to a consideration of the proportion of obvious waste, the smell of the material and its palatability to stock, it has been usual in the past to consider the chemical composition of silage from the point of view of the usual food-stuffs' analysis. This is not enough if an opinion is to be given as to the changes which have taken place during making. In the preparation of the sample for analysis, particularly in drying it, there are losses of volatile products, and these are usually not allowed for in the calculation of the results. The complete foodstuffs' analysis of a sample of silage does give important information, but it should be fortified by determinations of the volatile constituents which may be an important indication of the true value.

To obtain an indication of the nutritive value of the silage, it is possible to use the figures for the crude composition, but even these may prove to be very misleading. As an example, a digestibility trial was carried out recently on a sample of stack silage which had heated badly, reaching a temperature of over 170° F. The results are compared in Table I with an average sample of tower silage which had been made by the low-temperature process (80–100° F.).

Table I. *Composition and digestibility of overheated stack silage and average quality tower silage*

	Stack silage		Tower silage	
	Composition % of dry matter	Digestibility %	Composition % of dry matter	Digestibility %
Crude protein	15.33	35.26	14.78	67.70
Ether extract	1.51	48.25	4.33	77.68
N-free extractives	39.40	48.42	42.71	71.73
Fibre	31.69	52.72	28.28	77.26
Ash	12.07	—	9.90	—
"True" protein	9.40	0.00	7.82	49.55

Despite the lower crude-protein content of the tower silage, it is obvious that the digestible nutrients are considerably higher. On the

crude analysis the stack silage would, however, have been given the advantage. The high temperature in the stack has reduced the digestibility of all the constituents very considerably, and the "true" protein was entirely indigestible.

It is, of course, impossible to carry out a metabolism trial on every sample, and indeed, where the temperature has been adequately controlled, depression of the digestibility is not to be expected, and the ordinary foodstuffs' analysis will give a useful indication of the feeding value of the material. The colour of the sample is a useful index; a dark-coloured silage, with evidence of charring and high temperature, should be regarded with suspicion, and due emphasis laid on the possibility that the analytical values will tend to give too high a value when the nutritive value is under consideration.

#### MOISTURE CONTENT OF SILAGE

The moisture content of silage is a point of great importance. Amos & Woodman<sup>(5)</sup> have stressed this fact, and it is probably the most important determination where a knowledge of the feeding value is concerned. Since silage is a watery food, minor variations in moisture content may make appreciable differences in the actual feeding value. The moisture content of grass silage is in the neighbourhood of 75 per cent. If this should alter to 80 per cent, and this difference may often be found within the same silo, the dry matter is reduced from 25 to 20 per cent. To supply the same quantity of nutrients, a ratio of 40 lb. of silage with 75 per cent moisture would have to be increased to 50 lb. to allow for such a change in moisture content.

#### METHODS OF ANALYSIS

The determinations made on a sample of silage should be directed to obtaining information on the dry-matter and nitrogen contents, the acidity, the degree of protein decomposition, and the formation of organic acids. Given these figures, it is possible to comment on the quality of the silage, and to ascribe to it a nutrient content which can be used as a basis for determining its use in the ration of farm stock. General observations as to colour and smell, though useful, are not sufficient. Where more complete information is required, the ordinary foodstuffs' analysis can be carried out on the dried sample.

## SAMPLING

Where a crop has been chaffed, the sampling is not a very difficult process. Modern work has, however, resulted in the ensilage of crops without cutting, and it becomes a difficult matter to sample properly. The practice at this Station has been to take fairly large samples and cut these up into short lengths. A very useful laboratory chaff-cutter has been obtained from Messrs Gerhardt, Bonn-am-Rhein, at a reasonable cost. This will enable samples of 2-3 lb. in weight to be cut into short lengths very rapidly. This is most important where samples of mixed herbage are concerned. The whole sample can be chaffed rapidly, and is then mixed thoroughly, and the requisite sub-samples are drawn from the well-mixed mass. This chaff-cutter has very greatly simplified the work with silage, and can be recommended.

## MOISTURE CONTENT

Two samples of 200 g. each are drawn from the well-mixed sample, and dried at 98° C. in a steam oven equipped with an efficient air-circulation system, the drying process taking about 4 hours. The samples are weighed after being in the oven 3-3½ hours, replaced, and then reweighed at ½-¾ hour intervals until the weights are constant. To obtain the true dry-matter value, it is necessary to correct for the volatile constituents lost during the drying process. This can be done on the assumption that the loss of volatile bases and organic acids is complete, but as will be seen later, this is not correct. To obtain a true value, it is necessary to estimate volatile bases and volatile acids in the fresh and the dried sample, and work out from this the percentage lost on drying, and correct accordingly. In the later part of this paper the dry-matter values have been corrected on the basis of the losses of volatile substance, as summarized in Tables II and III.

## THE LOSSES OF VOLATILE ACIDS DURING DRYING

The examination of 67 samples of silage for total volatile acid content before and after drying, showed that the losses of volatile acid ranged from 50 to 92 per cent. The figures are summarized in Table II.

Table II. *The losses of volatile acids on drying samples of silage at 98° C.*

No. of samples examined	Volatile acid content of fresh silage (as acetic acid) %	Loss of volatile acid %	
		Mean	Range
10	0-0.49	68.1	50.1-78.4
31	0.50-0.99	77.7	55.2-92.2
21	1.00-1.49	75.9	59.5-91.3
5	1.50-1.99	84.2	73.6-90.2

It will be seen that for most samples of silage, the loss and therefore the correction will be about 77 per cent of the original acid. The losses of volatile bases will be dealt with later.

#### CRUDE PROTEIN

This is estimated on the dried material in the usual way by the Kjeldahl process, selenium being used as a catalyst<sup>(6)</sup>. The value for total nitrogen thus obtained is multiplied by 6.25, the factor usually adopted for the conversion of total nitrogen to crude-protein values.

As with the dry-matter determination, the crude-protein value must be corrected for volatile nitrogenous substances. This correction is determined from the volatile base figures, but here again it is necessary, to obtain the correct value, to know the percentage of the volatile bases which are actually lost on drying. For general purposes it can be assumed without great error in the crude-protein value that there has been a total loss of the volatile bases in the drying process, though, as will be seen later, this gives slightly too high a value to the corrected crude-protein figure.

#### PRODUCTS OF THE DECOMPOSITION OF THE CRUDE PROTEIN

*Ratio of "true" to crude protein.* The most usual measure of the protein breakdown is the ratio of total nitrogen to nitrogen precipitable by copper, which is often called "true" protein nitrogen. The copper precipitable nitrogen is usually estimated by the method first put forward by Stutzer<sup>(7)</sup> or Barnstein's modification<sup>(8)</sup>. Whilst this gives some approximation to the degree of breakdown, and may be used for some purposes, it is not a true measure, since the determinations are usually carried out on the dried material. The drying of the sample at 100° C. results in a variable loss of the volatile bases produced by the protein breakdown. Edin & Sandberg<sup>(9)</sup> give this loss on drying at 3.1, 14.5, 9.1 and 17.5 per cent of the total nitrogen on four cases they investigated. In the present investigation the losses were determined in 67 cases. The percentage losses increased with increasing amounts of volatile base present in the silage, but the figures showed considerable variation. The variation was probably mainly due to differences in the times of drying, but it is also possible that some of the volatile base was present in a combined state, and therefore less volatile.

A summary of the loss figures is given in Table III.

This loss of volatile bases decreases the figure for crude protein, and raises the proportion of "true" protein nitrogen in the total nitrogen.

Table III. *The losses of volatile bases on drying samples of silage at 98° C.*

No. of samples examined	Volatile base content of fresh silage % (calculated as crude protein)	Loss of volatile bases %	
		Average	Range
26	0 -0.49	32.2	0 -65.5
32	0.50-0.99	56.9	15.0-90.8
9	1.00-2.09	80.6	61.9-93.7

## WATER-SOLUBLE NITROGEN

Virtanen(10) measures the degree of protein degradation by the increase in water-soluble nitrogen in the product as compared with the fresh crop.

*Method*

25-30 g. of finely chaffed silage are thoroughly ground with water in a mortar, and quantitatively transferred to a measuring flask of 100 c.c. capacity. An addition of 0.5 c.c. of formalin is then made, and the flask filled up to the mark with distilled water. The flask and its contents are then shaken well, and kept overnight in an ice-chest. The solution is filtered through cloth, with the aid of a filter pump, and centrifuged. The nitrogen content of the clear solution is determined in an aliquot by the Kjeldahl method.

The soluble nitrogen consists chiefly of the non-protein nitrogenous substances, and should agree fairly well with the difference between the total nitrogen and the nitrogen precipitable by copper.

Table IV. *Comparison of water-soluble nitrogen and nitrogen non-precipitable by copper, in silage*

Water- soluble protein		Non- protein		(1) - (2)		(1)		(2)		(1) - (2)		(1)		(2)		(1) - (2)	
N	N	(1)	(2)	(1) - (2)	(1)	(2)	(1) - (2)	(1)	(2)	(1) - (2)	(1)	(2)	(1) - (2)	(1)	(2)	(1) - (2)	(1) - (2)
.332	.267	.065	.351	.331	.020	.328	.281	.047	.347	.278	.069	.293	.230	.063	.251	.217	.034
.309	.281	.028	.251	.217	.034	.238	.190	.048	.511	.473	.038	.341	.270	.071	.303	.259	.044
.356	.285	.071	.303	.270	.033	.367	.297	.070	.310	.302	.008	.316	.284	.032	.318	.270	.048
.328	.290	.038	.335	.295	.040	.508	.424	.084	.333	.286	.047	Av.		.342	.293	.049	

The water-soluble nitrogen exceeds the non-protein nitrogen by an average of about 17 per cent, which demonstrates that some of the water-soluble nitrogenous substances, probably the amino acids, are precipitable by copper.



The water extracts on which these figures are based were prepared by the method described later for the estimation of volatile bases and acids and amino acids by the Foreman method, and not by the method described by Virtanen.

#### VOLATILE BASES, AMINO ACIDS AND ORGANIC ACIDS

The most convenient analytical method for comparative purposes has been that of Foreman<sup>(11)</sup> as modified by Woodman<sup>(12)</sup>. This method gives a value for volatile bases which is a useful measure of the ultimate degradation products, and also a value for amino acids, which affords a means of identifying another group of nitrogenous compounds resulting from the breakdown of the protein. The total volatile acids are also estimated, but the method does not afford a separate measure of the combined and free acids or any differentiation of the various individual volatile acids present. In addition, an approximate measure of the lactic acid content can be obtained by calculation.

#### *Method*

*Extraction.* To 100 g. of finely chopped silage is added 500 c.c. of distilled water in a 1 litre cylinder. The extraction is then carried out either by shaking in an end-over-end shaker for 4 hours, the method usually adopted at this Station, or by allowing to stand for 24 hours with occasional shaking. The liquid is then poured through a coarse cloth, and the silage pressed by hand. The total extract is stirred thoroughly and then filtered, the filtrate being discarded until it comes through perfectly clear. Filtration is slow, but it is usually necessary to collect only about 300 c.c. for the usual estimations.

60 c.c. of the extract are placed in a 200 c.c. measuring flask and filled to the mark with neutral alcohol. The solution is filtered and used for the following estimations.

*Total acidity.* To 10 c.c. of the filtered aqueous-alcoholic extract is added 50 c.c. of neutral alcohol. The solution is then titrated with  $N/10$  NaOH, using phenolphthalein as indicator.

This figure gives a measure of all the acidic groups present in the solution, the basic groups being bound by the strongly alcoholic solution.

*Volatile bases.* To 50 c.c. of the aqueous-alcoholic extract in a 250 c.c. distilling flask is added an amount of  $N/10$  NaOH sufficient to react with all the acidic groups present (total acidity value  $\times 5$ ). The solution is then vigorously steam distilled for 7 min., the distillate being led into a

known volume of  $N/10$  sulphuric acid. The excess acid is then titrated with  $N/10$  NaOH, using Alizarin red as indicator, the difference being the amount used up by the volatile bases.

*Amino acids.* The residue in the flask develops alkalinity due to the release of the basic amino groups on the removal of the alcohol. The titration of this alkalinity with  $N/10$   $H_2SO_4$ , using phenolphthalein indicator, is therefore a measure of the amino acids.

*Volatile acids.* To the neutral residue in the flask is added sufficient  $N/10$   $H_2SO_4$  to release all the acids present. The total acid added, including that used for neutralizing the amino groups, should be equivalent to the amount of  $N/10$  NaOH added before the removal of the volatile bases. The solution is then steam distilled, the distillate being collected in quantities of 250 c.c. When the quantity of volatile acids present is of a low or medium order, 500 c.c. of distillate are collected, but when the acid content is high, the collection of 750 c.c. is advised. The volatile acids are titrated with  $N/10$  NaOH, using phenolphthalein.

*Calculation of results.* The assumption is made that the weight of liquids present in the silage is equivalent to an equal weight of water. The total volume of original extract available therefore from 100 g. fresh silage is  $500 + (100 - \text{dry-matter content})$  c.c.

Then if the total acidity titration figure is  $x$ , the total acidity as a percentage of the fresh silage is

$$\frac{20x}{60} (500 + (100 - \text{dry-matter content})).$$

For the other values, titration value  $y$ , the calculation is

$$\frac{4y}{60} (500 + (100 - \text{dry-matter content})).$$

*Residual acidity.* The total acidity being a measure of all the acidic bodies present, it is possible to calculate the acidity due to non-volatile acidic bodies, other than amino acids, by subtracting the sum of the amino acids and volatile acids from the total acidity. It will be seen later that this value approximates, as would be expected, to the lactic acid content of the silage except when the silage is made by the addition of mineral acids.

*Amino acids as estimated by the Van Slyke method.* A number of silages were examined for amino acid content by the Van Slyke method, in addition to the Foreman method. It was found that the Van Slyke method gave consistently higher values. The reason for this was found to lie in the fact that nitrogen was formed during the reaction, not only

from the amino groupings but also from the ammonia of the volatile bases. If the ammonia was removed before the Van Slyke estimation, the amino acid values by the two methods agreed very well.

*Organic acids.* The organic acids can be divided into volatile acids, consisting almost entirely of acetic and butyric acid and non-volatile acids, of which lactic acid forms the major part. The Foreman method already described gives a figure for total volatile acids, and it has been usual to calculate this figure in terms of acetic acid. Unfortunately, it does not give any measure of the butyric acid present in the sample.

The lactic acid can be calculated from the residual acidity in the Foreman method, and, as will be seen later, with a fair degree of accuracy, except when mineral acids have been added in the making.

#### VOLATILE ORGANIC ACIDS

*Wiegner's method.* On the Continent the method in general use for estimating the volatile acids is that of Wiegner<sup>(8)</sup>. This gives figures for acetic and butyric acids, and is largely used as a basis for the classification of silage.

The Wiegner method, as used in this laboratory, is as follows: 100 c.c. of silage extract, prepared as described previously, 90 c.c. distilled water, 10 c.c.  $N/1$   $H_2SO_4$  and a few pieces of pumice are placed in a 750 c.c. conical flask, which is lagged from the level of the liquid to the neck with asbestos string. The flask is fitted with a two-holed rubber stopper equipped with a 100 c.c. separating funnel and a wide delivery tube leading to a Liebig condenser. Distillation is commenced, and the distillate is collected in a 100 c.c. cylinder. When 100 c.c. have collected, distillation is stopped without touching the gas flame, by the addition of 100 c.c. of distilled water through the separating funnel. Distillation soon recommences, and in this way three fractions of 100 c.c. of distillate are collected. The distillates are titrated with  $N/10$   $NaOH$ , using phenolphthalein, and if the titration values are designated  $T_1$ ,  $T_2$  and  $T_3$ , the formulae for the calculation of the acetic and butyric acid are as follows:

$$\text{Acetic acid} = 3.962 (T_2 + T_3) - 1.3724 (T_1).$$

$$\text{Butyric acid} = -1.992 (T_2 + T_3) + 2.0461 (T_1).$$

These values represent the acids present in 100 c.c. of silage extract, and the acids in 100 g. fresh silage are calculated by multiplying the values by  $\frac{500 + (100 - \text{dry-matter content})}{100}$ .

The above method estimates the total volatile acids present, free and

Table V. *Total volatile acids as determined by the Wiegner and Foreman methods. (Stated as percentages of acetic acid in the fresh silage)*

1) Foreman	(2) Wiegner	No butyric acid present					
		(1) - (2)	(1)	(2)	(1) - (2)	(1)	(2)
-87	0.81	+0.06	0.52	0.52	±0.00	0.25	0.21
-60	0.58	+0.02	0.45	0.45	±0.00	0.28	0.31
-73	0.65	+0.08	0.56	0.46	+0.10	0.40	0.33
-73	0.68	+0.05	0.42	0.42	±0.00	0.34	0.31
-10	0.07	+0.03	0.97	0.97	+0.00	0.35	0.35
-84	0.80	-0.05	1.28	1.30	-0.02	0.32	0.25
-88	0.83	+0.05	1.08	1.00	+0.08	0.26	0.25
-41	0.41	±0.00	1.00	1.00	±0.00	0.31	0.31
Mean difference = +0.02 ±0.03.							
1)	(2)	Butyric acid present					
		(2) - (1)	(1)	(2)	(2) - (1)	(1)	(2)
-51	0.47	-0.04	1.30	1.34	+0.04	0.31	0.27
-67	0.54	-0.13	1.30	1.81	+0.51	0.42	0.34
-31	1.21	-0.10	1.36	1.38	+0.02	0.54	0.45
-92	0.90	-0.02	1.83	2.04	+0.21	0.54	0.50
-92	0.90	-0.02	1.66	1.71	+0.05	0.62	0.51
-69	0.83	+0.14	0.91	0.90	-0.01	0.59	0.60
-14	1.46	+0.32	0.63	0.72	+0.09	0.68	0.55
-12	1.27	+0.15	1.00	0.93	-0.07	0.65	1.11
-00	1.01	+0.01	0.89	0.72	-0.17	0.71	0.59
-94	0.90	-0.04	0.70	0.60	-0.10	0.40	0.44
-92	1.03	+0.11	0.65	0.72	+0.07	0.65	0.58
-01	1.03	+0.02	0.39	0.31	-0.08	0.46	0.38
-19	1.47	+0.28	0.72	0.71	-0.01	0.80	0.83
-71	0.80	+0.09	0.17	0.19	+0.02	0.52	0.52
-10	1.18	-0.08	1.20	1.34	+0.14	0.47	0.36
-55	0.56	+0.01	0.77	0.72	-0.05	0.50	0.39
Mean difference = +0.01 ±0.13							

The figures cover a wide range, the samples with high values containing fairly large amounts of butyric acid.

combined. In estimating the free acids alone, the 10 c.c.  $N/1$   $H_2SO_4$  are omitted, 100 c.c. distilled water only being added to the silage extract.

The agreement between the values for volatile acids by the Wiegner method and the Foreman method is satisfactory, as can be seen from Table V. In the course of the work on silage 118 samples were examined for volatile acids by the two methods. The values for the Foreman estimation were calculated as acetic acid in the usual way, and the butyric acid values in the Wiegner process have been calculated as acetic acid and added to the estimated acetic acid figures. In the first part of the table are summarized the values for all the silages containing no butyric acid, and in the second 62 samples containing both acetic and butyric acids. Samples containing less than 0.1 per cent of butyric have not been included in the table.

There is little difference between the values obtained by the two methods when butyric acid is absent. In the presence of butyric acid the differences are more marked, and the figures when plotted show that the Foreman method tends to give slightly higher values than the Wiegner method when the total volatile acid content is about 0.5 per cent. When the acid content approaches and exceeds 1.0 per cent, the reverse applies. The range of difference between the 62 samples in the two series, when butyric acid is present, is 0.00 to 0.51, but only five samples gave differences of over 0.20, while 44 samples gave differences of 0.10 and under.

#### NON-VOLATILE ORGANIC ACIDS

The chief acid here is lactic acid, and as has been stated, it can be approximately estimated by calculation from the residual acidity, as found in the Foreman method, in silages where no mineral acid has been added. The actual determination of lactic acid has been made by the method worked out by Hirsch-Kauffmann<sup>(13)</sup>, based on the principle of Furth Charnass. The practical details of the estimation are described, together with certain modifications, by Karström<sup>(14)</sup>.

The estimated lactic acid figures compared with the calculated values of various types of silage are given in Table VI.

The agreement in the ordinary silage is not too good, but in the silages made with added molasses and whey, where the lactic acid content is reasonably high in most cases, it is much better. Where acid has been added in the filling of the silo, the calculated figure, as might be expected, is very much higher, and the greater the addition, as in the A.I.V. process, the greater this difference becomes. It would appear that for ordinary silage, with or without added carbohydrates, the residual

acidity calculated as lactic acid will give a reasonably accurate measure of the lactic acid present, and will be sufficiently close for work involving an examination of large numbers of samples. Where mineral acids have been added, however, it is necessary to estimate the lactic acid directly if this be considered necessary. In general, it is not an essential determination, and as will be seen later, the hydrogen-ion concentration is the most useful index to quality.

Table VI. *The relationship between the estimated lactic acid in silage and the value calculated from the residual acidity in the Foreman method (percentage fresh silage)*

Type of silage	No. of samples	Estimated		Calculated		Difference (C - E)	
		Av.	Range	Av.	Range	Av.	Range
Silage with added acid (pH 4.5)	12	0.65	0.33-1.09	1.09	0.25-1.78	+0.44	-0.08 to +0.91
Silage with added acid (A.I.V. pH below 4.0)	13	0.75	0.30-1.17	1.43	0.80-2.10	+0.68	+0.15 to +1.15
Ordinary silage	8	0.54	0.16-1.15	0.73	0.09-1.74	+0.18	-0.13 to +0.63
Molasses	12	0.92	0.38-2.03	1.07	0.52-2.73	+0.15	-0.14 to +0.70
Whey	2	0.40	0.26-0.53	0.34	0.28-0.40	-0.06	-0.13 to +0.02

#### ACIDITY OF THE MASS

The total acidity of the fodder can be obtained by the Foreman method, but is not the best criterion. The hydrogen-ion concentration is the most useful figure, and the low degree of dissociation of the organic acids is the reason for the difference in rate of acidification of the mass as compared with processes such as the A.I.V., which depend upon the addition of readily dissociated mineral acids such as hydrochloric. This indeed accounts for the advance of this process over previous attempts using organic acids, in which the amount needed to bring about the correct reaction in the mass was, relative to the amount of mineral acids needed for the same purpose, extremely high. The total acidity bears no mathematical relation to the pH of the mass, as is seen from Table VII.

Table VII. *pH and total acidity of the mass (Foreman method) of ordinary grass silage and grass silage made with added molasses and added acid*

pH	Total acidity, as c.c. N/10 per 100 g. fresh silage		
	Ordinary silage	Added molasses	Added acid (A.I.V.)
Under 4.00	498.9	465.9	318.0
4.00-4.49	363.7	431.0	291.6
4.50-4.99	288.4	322.2	229.8
5.00 and over	270.2	226.2	—

Within the effective range for A.I.V. fodder—under  $pH$  4.0—the total acidity for the same  $pH$  value is considerably less than in ordinary silage with or without added carbohydrates. For values above  $pH$  4.0 the silage with added acid becomes more like ordinary silage, and the difference in the total acidity values is not so striking, though the ordinary silage values still remain somewhat higher. The  $pH$  is determined electrometrically, using quinhydrone and a calomel cell, on juice expressed from the fresh silage. It would seem from a consideration of all the facts, that the Foreman method, as modified by Woodman reinforced by  $pH$  values, is the best process for use in the classification of silages. It gives a value for the volatile bases, which is a measure of the degree of protein breakdown to compounds which are of debatable value in animal nutrition. The amino acid figure gives information as to intermediate changes. In good silage the volatile acid determination by this method is a useful index of the degree of change suffered by the non-nitrogenous fraction of the silage. If the value for volatile acids thus obtained, and calculated as acetic acid, is high, it may be necessary to fractionate the volatile organic acids according to Wiegner's method. In general, however, a low total volatile acid figure denotes a low butyric acid content, which may be ignored unless the silage is to be fed to cows producing milk used for the manufacture of certain types of cheese, where the presence of butyric acid may lead to trouble.

In general, it is not the butyric acid which is a danger, but a large amount of this acid is often associated with undesirable changes in the protein due to the action of the anaerobes. Putrefactive changes are noticeable in extreme cases, and may cause trouble when fed to stock. If due care be taken to prevent tainting of the milk by exposing it to the effect of silage in the cowshed, no difficulty is to be expected in feeding silage containing butyric acid to any class of stock; it will still be eaten readily.

No limit can be set to the percentage of butyric acid which is permissible, but where it exceeds 0.75–1.00 per cent of the fresh silage, due care should be taken in its use, and it would be better fed out of doors. The aim of silage making, however, should be to reduce the percentage of butyric acid present to the lowest possible level, and no appreciable amount should be found in a good sample of silage.

Though alcohol is formed as an intermediate product in the silage process, this constituent has not been estimated. The amounts of alcohol present in silage as stated in the literature vary, but according to Mangold (15) are of the order of 0.2–0.3 per cent on the fresh material.

When the ordinary foodstuffs' analysis is carried out on samples of dried silage, certain corrections are necessary. The crude-protein values have to be corrected for volatile bases, lost in the drying process, on the lines already discussed. The ether extract also requires correction, since it would normally include the volatile organic acids lost on drying, and due allowance should be made for this. This is a point worthy of note; the ether-extract values in silage are always high as a result of the inclusion of all the organic acids not lost during drying, since they are extracted by the solvent. In calculating the nutritive value of silage this has to be taken into account, since the organic acids may often form up to 6 per cent of the dry matter, and will, on correction, increase the figure considerably. These compounds, however, do not possess the calorific value of the remainder of this fraction and, in fact, the volatile acids will only supply a fraction of the energy of the original carbohydrates.

#### MATERIAL EXAMINED

During the last three years a very large number of samples of silage have been obtained from different parts of the country. The method of obtaining the samples was standardized and proved successful. After carefully clearing a surface on the top of the silo by removing a few inches' depth of silage over the spot to be sampled, a biscuit tin was placed mouth downward on the freshly exposed surface. A cut was made round the sides of the biscuit tin with a hay knife, and the tin forced down the cuts. The tin thus enclosed an undisturbed block of silage, which fitted tightly into the tin, since the cuts were made on the outside. The tin was forced down till the bottom was level with the surrounding silage, a hand was slipped down the side and placed under the mouth of the tin, which was then lifted out with its contents. The lid was put on the tin immediately, tied down tightly, and sealed with adhesive tape. The samples all travelled satisfactorily, there was never any sign of mould in any part of the tins, which were at times 3 days in transit. The sample taken for analysis was obtained from the centre of the tin, the outer edges being discarded and the remainder all put through the chaff-cutter.

The samples have been taken in the main from small wooden silos of the type advocated by Virtanen<sup>(4)</sup>, but a very large number have been taken from tower silos, and one or two from stacks. As has already been stated, the most important point about silage is the reaction of the mass, and the simplest way to classify the different silages is not according to



the container used, but they should be grouped by the process used and the *pH* of the sample.

In all, some 293 samples of silage have been examined, divided as follows:

Table VIII. *Details of samples of silage examined*

	Grassland herbage	Silage mixtures	Sugar-beet tops	Potatoes
Ordinary silage	65	14	3	1 (steamed)
With added molasses	38	4	—	—
With added whey	4	—	—	—
With added salt	1	—	—	—
With moderate additions of mineral acid (to <i>pH</i> 4·5)	7	—	—	—
With added acid (A.I.V. process to <i>pH</i> 3·0–4·0)	143	6	6	1
Total	258	24	9	2

The method of making the ordinary silage varied somewhat, and was representative of silage as generally made in this country.

The rate of addition of molasses was 1–2 lb. per 100 lb. of fresh grass, a dilute solution being sprinkled on by some convenient means. Where whey was used, the amount was calculated to give an addition of 1 lb. of lactose per 100 lb. of the fresh grass. The salt was added at the rate of 14 lb. per ton. The silage made with moderate amounts of mineral acid, with or without molasses, was designed to produce a *pH* of 4·5 in the mass immediately, on the lines of the Defu process<sup>(16)</sup>.

The A.I.V. fodder was made by the process introduced by Virtanen (2, 4, 10), the amount of acid recommended by him being applied in all cases.

The sugar-beet top silage was made in simple wooden containers in all but two cases, one with added acid, one without, in which clamps were used. The ordinary process potato silage was made in a pit from steamed potatoes, the other from raw potatoes with added acid, also in a pit. A number of samples of silage made from arable crops was also examined. In the main, these consisted of vetches and oats, but in some cases peas and beans were also included. Otherwise they were dealt with exactly in the same way as the grass silage.

Notes were taken on the colour, appearance and smell of the samples. The colour of the grass silages at the lowest *pH* values was usually a golden yellow to a greenish yellow, the colour getting darker with increasing *pH*. No samples showed any sign of charring. The colour of the silage where mineral acids were added was usually somewhat more yellow than in its absence, and the gradation in colour was not so marked.

The smell of the ordinary silage was faintly acid at the greatest acidity, becoming more strongly so at pH 4.50 and over, when the smell of acetic acid became stronger. Butyric acid could be smelt in some of the samples in this latter class, and above pH 5.00 the butyric acid was obvious, one or two samples having an extremely strong smell of this acid. In two or three samples where butyric acid was high, putrefactive changes were obvious to the nose.

The molassed silage was extremely pleasant to the smell at all acidities, with the characteristic sweet smell of the molasses. No sample showed any obvious smell of butyric acid. The silages made with added whey and with salt all had a slightly acid but not unpleasant smell, the salted silage alone having a decided vinegary smell.

The smell of the A.I.V. fodder was pleasant at the higher acidities, and some of these samples had a strong and pleasant smell of esters of the fatty acids. In one or two cases the smell was strong enough to be irritating. As the acidity fell, the silages of this process became identical with the ordinary silages, though even at the lowest acidity there was never the strong butyric acid smell which one or two of the ordinary silages possessed. The forage crop samples were very similar to those made from grassland herbage, but were apt to be darker in colour and more strongly acid in smell.

In all cases the original plants could easily be identified, and there was no obvious breakdown of the tissues.

The sugar-beet tops treated with acid by the A.I.V. process were remarkable, in that the leaf had been retained and had kept its form, whereas in the ordinary silage of this crop the whole mass had lost its form and the leaves were indistinguishable. The addition of acid removed the distinct smell of putrefaction and butyric acid so noticeable in the ordinary beet-top silage. The steamed-potato silage had a pleasant weakly acid smell and was very dense, whilst the raw potatoes treated with mineral acid had a distinctly acid smell.

#### CHEMICAL CHARACTERISTICS

The determinations made on each sample were pH, total acidity, crude protein, "true" protein, volatile bases, amino acids, total volatile acids and dry matter. In addition, 118 samples were examined by the Wiegner method for acetic and butyric acid. It was found that within any one process the silages of the same pH reaction showed similar values for the main decomposition products.

The crude-protein values have been corrected for nitrogen lost on

drying by using the respective loss figures, as quoted in Table III, whilst the dry-matter values have been corrected for the loss of volatile acids and volatile bases, using the figures for percentage loss of these constituents summarized in Tables II and III.

The ordinary silage and silage made with added molasses are classified into four groups, *pH* under 4.00, 4.00–4.49, 4.50–4.99, 5.00 and over. The silages made with added acid according to the A.I.V. process are classified in a similar manner, except that they start at *pH* 3.00 and under, rising by intervals of 0.5 *pH* up to 4.50, the fifth class being *pH* 4.50 and over. This refers to samples of silage made from grassland herbage and silage crops, which are classed separately. No one of the other processes or crops included a sufficient number of samples to justify any such subdivision.

#### DRY MATTER AND ACIDITY OF SILAGE

The average dry-matter content, *pH* reaction and acidity of the silages from each of these groups are summarized in Table IX. The total acidity is split up into that due to the volatile acids, the amino acids and the residual acidity, and the figures are given in terms of the titration values per 100 g. of fresh silage.

The dry-matter values do not show any particular trend, the average value for all the grass silages being 22.9 per cent, whereas for the forage crops it was 27.2 per cent. It is, however, noticeable that where the value falls to 20 per cent the *pH* value is high, and as will be seen from Table XIV, the butyric acid is very high; evidence of anaerobic fermentation.

The ordinary grass silage samples show that the greatest number of samples (46 per cent) had a *pH* value between 4.5 and 5.0, the majority (21 per cent) of the remainder lying between the values of 4.0 and 4.5. Where molasses was added better results were obtained, the greater part of the samples (47 per cent) showing *pH* values between 4.0 and 4.5, and an appreciable number (29 per cent) had a *pH* lower than 4.0. The small number of samples made with added whey showed a very satisfactory average *pH* value of 3.86, no one of them exceeding 4.12. The sample with added salt was also excellent in this regard. Where moderate additions of acid were made, with or without added sugar, the *pH* of the product was reminiscent of the majority of the samples made with added molasses, and would be classed as good silage.

The A.I.V. process produced excellent silage, 69 per cent of the samples falling below the desired level of *pH* 4.0. The greater majority

Table IX. *Dry matter, pH and acidity of grass and other silages*

Type of silage	No. of samples averaged	Dry matter %	pH	Total acidity c.c. N/10*	Amino acids c.c. N/10*	Volatile acids c.c. N/10*	Residual acidity c.c. N/10*
1. Ordinary silage							
A. Grassland herbage							
pH under 4.00	4	24.9	3.94	498.9	130.3	135.7	232.9
pH between 4.00 and 4.49	20	28.2	4.24	363.7	85.7	142.0	136.0
pH between 4.50 and 4.99	30	22.7	4.76	288.4	72.0	150.1	66.3
pH 5.00 and over	11	20.2	5.21	270.2	52.6	199.3	18.3
B. Forage crops							
pH under 4.00	4	26.2	3.81	500.4	130.3	121.3	248.8
pH between 4.00 and 4.49	7	30.9	4.29	363.9	96.0	152.3	115.6
pH between 4.50 and 4.99	3	32.7	4.83	363.0	107.4	178.2	77.4
2. Silage made with added molasses							
A. Grassland herbage							
pH under 4.00	11	25.9	3.88	465.9	112.0	115.0	238.9
pH between 4.00 and 4.49	18	25.5	4.19	431.0	115.4	139.3	176.3
pH between 4.50 and 4.99	6	23.1	4.67	322.2	94.8	136.7	90.7
pH 5.00 and over	3	23.2	5.58	226.2	78.8	110.5	36.9
B. Forage crops							
pH between 4.00 and 4.49	3	30.7	4.10	399.0	93.7	164.9	110.4
pH 5.00 and over	1	22.3	5.37	348.1	61.7	286.4	0.00
3. Silage made with added whey (fresh, or solutions of concentrated whey)							
Grassland herbage	4	26.5	3.86	432.4	113.1	87.1	232.2
4. Silage made with added salt							
Grassland herbage	1	37.3	3.85	376.0	43.5	180.0	152.5
5. Silage made with moderate additions of mineral acid, with and without molasses							
Grassland herbage	7	25.0	4.32	390.2	115.4	130.2	144.6
6. A.I.V. fodder. Mineral acids added in amounts designed to produce a pH of 3.0-4.0							
A. Grassland herbage							
pH under 3.00	13	25.4	2.49	282.1	38.8	59.5	183.8
pH between 3.00 and 3.49	21	21.4	3.29	274.3	53.7	57.1	163.5
pH between 3.50 and 3.99	65	23.6	3.73	339.3	74.3	81.7	183.3
pH between 4.00 and 4.49	30	21.9	4.17	291.6	70.9	103.9	116.8
pH 4.50 and over	14	19.7	4.81	229.8	57.1	116.8	55.9
B. Forage crops							
pH between 3.00 and 3.49	1	27.7	3.45	276.8	43.5	57.7	175.6
pH between 3.50 and 3.99	3	21.0	3.90	327.8	60.6	90.4	176.8
pH between 4.00 and 4.49	1	25.3	4.47	345.0	45.7	145.9	153.4
pH 4.50 and over	1	24.7	4.76	470.4	185.1	135.5	149.8
7. Silage made from sugar-beet tops							
Without any addition	3	29.3	4.16	362.9	78.8	134.5	149.6
With added acid (A.I.V. process)	6	25.3	3.93	309.4	67.4	101.4	140.6
8. Silage made from potatoes							
Steamed. No addition	1	29.1	4.07	505.6	37.7	271.4	196.5
Raw. With added acid	1	44.5	4.83	259.8	81.1	178.7	0.0

\* Stated as c.c. N/10 per 100 g. of fresh herbage.

of the samples (45 per cent) fell between the values of  $pH$  3.5 and 4.0. Of the 31 per cent of samples with a  $pH$  value over 4.0 some 21 per cent showed an average value of 4.17, a value not far removed from the desired level and below the figure given by Virtanen for the repression of butyric acid fermentation ( $pH$  4.2). The silage made from forage crops gave results which were generally in line with those noted above for grass silage.

The addition of acid to sugar-beet tops resulted in a lower  $pH$  value than where none was added, but even here the average value is not too high.

The number of potato silages examined was not large enough to enable any comparison to be made, and, furthermore, the steaming of the control potatoes allowed of better packing, whereas the acid would tend to run fairly freely from the ensiled raw potatoes.

The titration values for total acidity agree in order with the  $pH$  values within each process. Mention has already been made of the lower values in the A.I.V. fodder in relation to  $pH$ , and the reason for this has been discussed. For  $pH$  values under 4.0 the ordinary and molassed silages show residual acidity values, due in this case chiefly to lactic acid, which are appreciably higher than is the case for A.I.V. fodder. Above this  $pH$  level, however, there is fair agreement in the values for residual acidity. The amino acids and volatile acids are better discussed separately, and will be dealt with later.

These remarks apply with equal force to the samples of silage made from forage crops.

#### PROTEIN BREAKDOWN OF SILAGE

In Table X the values for total nitrogen and its constituent fractions have been summarized.

The first point to notice is the relatively low value for crude protein content in the majority of the samples. This is very striking, the average crude-protein value for all the samples of grass silage being 15 per cent of the dry matter, and for the forage crop 14.3 per cent. This is evidence of practice in this country where the crop is cut for silage at the pre-hay stage some 3–4 weeks before normal hay time, and when the crop can be cut and handled with ordinary farm machinery. Piraux *et al.* (17) show in their series of analyses that the average crude-protein content of 30 samples of silage made from grass was 14.6 per cent of the dry matter. It should be realized that this is not a necessary limitation of the silage process, as material of higher crude-protein content can be made into

good silage. There are only two groups of silage in the summarized tables which average over 17 per cent of crude protein in the dry matter. In the molassed silage there is a group of three silages, but the silage produced here was the poorest, and the average is brought up by one sample which contained about 23 per cent of protein. The seven silages made with moderate amounts of added acid have given a much better product. It is not fair to draw final conclusions for such small groups, and all silages containing over 17 per cent of crude protein have been extracted from the individual results, and have been summarized separately. These will be discussed later. The samples of sugar-beet top silage show a surprisingly high crude-protein content, and compare favourably with the average of all the other silages in this regard.

The "true" protein values all show evidence of considerable breakdown of protein. This is seen more clearly from the figures for the percentage of "true" protein in the total protein. Greenhill<sup>(18)</sup> has shown that grassland herbage normally contains some 80-90 per cent of the crude protein in the form of "true" protein. An examination of the analytical results obtained by Amos & Woodman<sup>(19)</sup> with green oats and tares shows that the "true" protein forms 70-80 per cent of the crude protein present.

In the grass silages examined in the present study, the percentage of "true" protein is reduced to 40-54 per cent in ordinary and molassed silage, whereas in the A.I.V. process it is 60-67 per cent. This upholds the claims of Virtanen, to the extent that protein breakdown is reduced, though not prevented, by the addition of mineral acids and the rapid reduction of the mass to a relatively low pH value. The control of the breakdown is not as complete as might have been expected; Spildo<sup>(20)</sup> has also found this breakdown to occur.

The degree of breakdown of the "true" protein is not, however, of such importance as the nature of the breakdown products. The most important figure in this regard is the volatile base content, and Virtanen<sup>(10)</sup> has stressed the importance of the percentage of ammoniacal nitrogen in silage, particularly when stated as a percentage of the total nitrogen present. The volatile base figures obtained by the Foreman method are invaluable in this regard, and are strictly comparable with figures for ammoniacal nitrogen obtained by direct distillation. They are always determined on the fresh silage, but it is imperative for comparative purposes to consider them on a dry-matter basis. This is quite legitimate, as the crude-protein figures have been corrected for loss of volatile constituents on drying. An examination of the figures shows

Table X. *The protein breakdown in grass and other silages*

Type of silage	No. of samples averaged	Crude protein % of dry matter	Percentage of crude protein as							Ratio Amino acids Volatile bases
			"True" protein % of dry matter	Volatile bases*		Amino acids*		Volatile bases + amino acid + "true" protein		
				In fresh silage %	In dry matter %	In fresh silage %	In dry matter %			
1. Ordinary silage										
A. Grassland herbage										
pH under 4.00 and 4.49	4	13.66	6.20	0.39	1.57	1.14	4.58	11.49	33.53	45.02
pH between 4.00 and 4.49	20	12.51	6.74	0.38	1.35	0.75	2.66	11.19	32.63	45.88
pH between 4.50 and 4.99	30	15.23	7.27	0.58	2.55	0.83	2.77	16.71	18.15	37.86
pH 5.00 and over	11	17.12	6.84	0.88	4.36	0.46	2.28	25.47	13.32	38.79
B. Forage crops										
pH under 4.00	4	13.21	5.20	0.33	1.26	1.14	4.35	9.54	32.93	42.47
pH between 4.00 and 4.49	7	14.30	6.82	0.51	1.65	0.84	2.72	11.54	19.02	35.56
pH between 4.50 and 4.99	3	12.57	5.56	0.52	1.59	0.94	2.87	12.65	22.83	35.46
2. Silage made with added molasses										
A. Grassland herbage										
pH under 4.00	11	15.10	7.66	0.28	1.08	0.98	3.78	7.15	25.03	32.18
pH between 4.00 and 4.49	18	14.81	7.14	0.33	1.29	1.01	3.96	8.71	26.74	35.45
pH between 4.50 and 4.99	6	15.67	7.54	0.46	1.99	0.83	3.59	12.70	22.91	35.61
pH 5.00 and over	3	19.09	9.54	0.63	2.72	0.69	2.97	14.25	15.56	29.81
B. Forage crops										
pH between 4.00 and 4.49	3	14.33	6.96	0.37	1.20	0.82	2.67	8.37	18.63	27.00
pH 5.00 and over	1	25.36	12.53	1.39	6.23	0.54	2.42	24.56	9.54	34.10
3. Silage made with added whey (fresh, or solutions of concentrated whey)										
Grassland herbage	4	14.07	6.03	0.21	0.79	0.99	3.74	5.61	26.58	32.19
4. Silage made with added salt										
Grassland herbage	1	11.84	7.00	0.46	1.23	0.38	1.02	10.39	8.61	19.00
5. Silage made with moderate amounts of mineral acid, with or without molasses										
Grassland herbage	7	19.96	9.65	0.50	2.00	1.01	4.04	10.02	20.24	30.26
6. A.I.V. fodder. Mineral acids added in amounts designed to produce a pH of 3.0-4.0										
A. Grassland herbage										
pH under 3.00	13	10.71	6.90	0.12	0.47	0.34	1.34	4.39	12.51	10.90
pH between 3.00 and 3.49	21	13.90	9.25	0.18	0.64	0.47	2.20	6.04	15.83	21.87
pH between 3.50 and 3.99	65	15.72	9.40	0.24	1.02	0.65	2.75	6.49	23.98	29.79
pH between 4.00 and 4.49	30	15.16	9.06	0.27	1.23	0.62	2.83	8.11	18.67	26.78
pH 4.50 and over	14	15.21	9.04	0.43	2.18	0.50	2.54	14.33	16.70	31.03
B. Forage crops										
pH between 3.00 and 3.49	1	10.03	5.99	0.12	0.43	0.38	1.37	4.29	13.66	17.95
pH between 3.50 and 3.99	3	13.98	7.61	0.16	0.76	0.53	2.52	5.44	18.03	23.47
pH between 4.00 and 4.49	1	12.81	6.42	0.39	1.54	0.40	1.58	12.02	12.34	24.36
pH 4.50 and over	1	18.70	9.73	0.42	1.70	1.62	6.56	9.09	35.08	44.17
7. Silage made from sugar-beet tops										
Without any addition	3	14.26	6.73	0.42	1.43	0.69	2.35	10.03	16.48	26.51
With added acid (A.I.V. process)	6	15.45	9.19	0.36	1.41	0.59	2.30	9.13	14.89	24.02
8. Silage made from potatoes										
Steamed. No addition	1	12.09	7.24	0.37	1.27	0.33	1.13	10.50	9.35	19.85
Raw. With added acid	1	14.05	8.27	0.62	1.39	0.71	1.60	9.89	11.28	21.28

\* Calculated as crude protein, i.e. volatile base and amino N  $\times$  6.25.

\* Calculated as crude protein, i.e. volatile base and amino N  $\times$  6.25.

first of all a definite relationship between the  $pH$  value and the percentage of the total nitrogen in the form of volatile bases, exactly as Virtanen says. This holds good for all the grass silages, and those made from silage crops, with but one exception, forage crops made by the A.I.V. process, where the least acid sample shows a relatively low percentage of volatile bases, but this is an isolated case.

As between the processes, it is obvious that in ordinary silage, even under the best conditions, volatile base formation is greater than in any other. This is markedly decreased where molasses or mineral acids are added. The lowest values are shown in A.I.V. fodder, whether made from grass or silage crops. Here the percentage of volatile base nitrogen does not exceed 6.49 per cent of the total nitrogen where the  $pH$  is under 4.0. For silage with added molasses for the same  $pH$  value the figure is only 7.15 per cent—no great difference—and between  $pH$  4.0 and 4.5, 8.71 per cent; the majority of these molassed silages falls within these two classes. Where, for some reason, the  $pH$  has not been reduced by the addition of presumably sufficient amounts of acid solution in the A.I.V. process—and a few such cases do occur in practice—the values for volatile bases do not differ appreciably from those in molassed silage of the same  $pH$  value.

As the volatile bases increase so, in the ordinary and molassed silage, do the amino acids decrease, the total breakdown of protein to these two forms being fairly constant in the silage with added molasses.

The formation of amino acids in A.I.V. fodder is markedly less than in any other form of silage making, increasing but slightly with the  $pH$ .

The sum of the volatile base, amino acid and “true” protein nitrogen accounts for the greater part of the total nitrogen of the silage, the difference presumably being due to more complex nitrogenous compounds intermediate between the amino acids and the protein. The forage crops show a somewhat greater proportion of this residue than the grass, and the molassed crops more than ordinary silage or A.I.V. fodder.

Piroux and his associates<sup>(17)</sup>, in their examination of a series of silage samples, determined the percentages of ammoniacal nitrogen in the products, and obtained the values for grass silage shown in Table XI.

If these figures are compared with the values obtained in the present investigation, there is seen to be good agreement, though the figures for cold-fermentation silage are rather high as compared with ordinary silage of  $pH$  4.5–5.0 (average 4.76).

De Ruijter de Wildt<sup>(21)</sup>, as a result of an examination of 83 samples of silage, has published an interesting table showing the relationship



Table XI. *Percentage of ammoniacal nitrogen as percentages of total nitrogen in grass silage (Piriaux et al. (17))*

	No. of samples	Average pH value	Ammoniacal N as % total N
Warm fermentation in towers	4	4.1	9.47
Warm fermentation in stacks	3	4.7	10.35
Cold fermentation	3	4.3	18.13
Cold fermentation with added molasses	2	3.9	9.77
Silage made with added acid	6	3.5	4.81
„ „	9	3.9	8.40

between the pH value and the percentage of total nitrogen in the form of ammoniacal nitrogen. The figures are reproduced below.

Table XII. *Relationship between pH reaction of grass silage and the ammoniacal nitrogen (de Wildt (21))*

No. of samples	Average pH value	Ammoniacal N as % of total N
9	5.08	27.4
16	4.16	12.3
21	3.79	8.3
27	3.51	5.9
10	3.18	3.9

These again show the same trend, and it is clear from all the results discussed that the pH of the silage is correlated with the degree of volatile base or ammonia formation. It is only when the pH of the mass rises above 4.5 that the formation of volatile bases becomes very high, and this is more marked with ordinary silage than any of the other processes. The difference between A.I.V. fodder and molassed silage at similar pH values is negligible, but the addition of acid results on the whole in a lower pH than molasses, and this confers a slight advantage on the former. The magnitude of this difference is only of the order of an increase from 6 to 8 per cent in molassed silage.

Amos & Woodman (19) in studying the changes which occur during the ensilage of oats and tares, have stressed the importance of the ratio of volatile base to amino acids, and state that in good silage the amino acids are in excess, whereas in bad silage the opposite may be the case. This is confirmed by the figures in the last column of Table X. These show for molassed silage that the formation of amino acids, in proportion to the volatile bases, is greater than in any other process.

The differences between the three main processes are seen clearly for grass silage in Fig. 1, where the values for volatile bases, amino acids and ratio amino acids/volatile bases have been plotted against pH. The shortcomings of the ordinary silage process, as measured by volatile

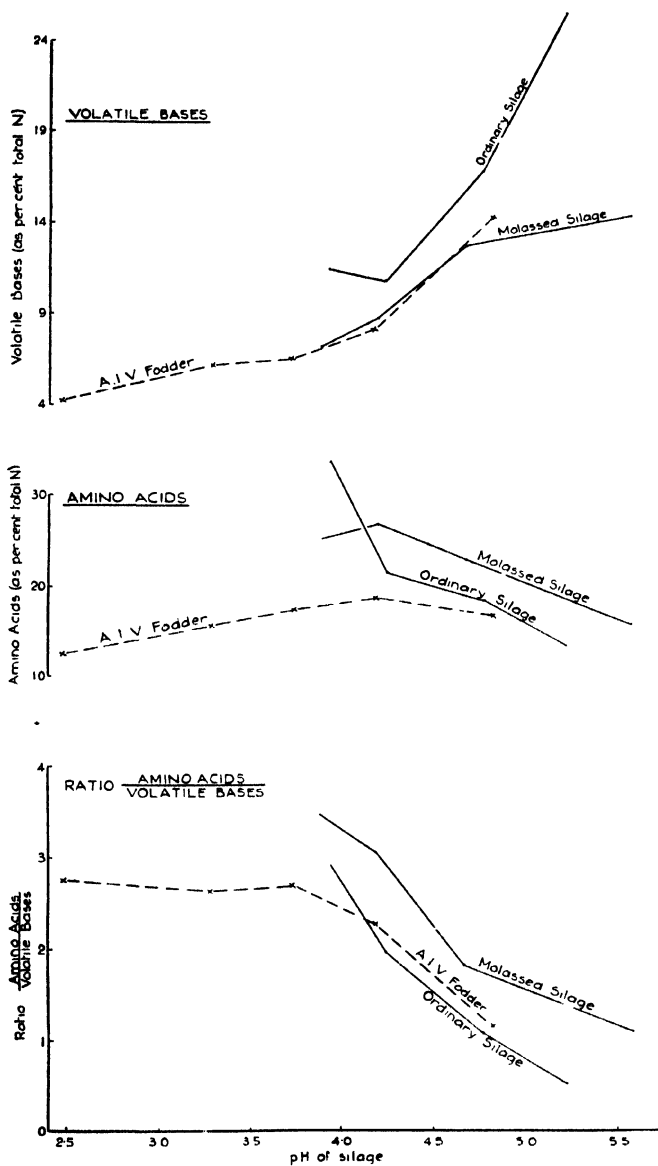


Fig. 1.

base formation, are evident, and increase with the *pH*. The values for A.I.V. fodder and molassed silage are indistinguishable over the range covered by both of them. The volatile base formation increases with the *pH* value, and the only advantage of the addition of mineral acids over molasses rests in the lower *pH* value obtained in some of the samples. Below *pH* 4.0 the rate of formation of volatile base does not increase so rapidly as it does above *pH* 4.0.

The addition of acid reduces amino acid formation, which increases slightly but gradually with the *pH* up to 4.0, and falls thereafter. The molassed silage shows the same tendency, but the percentage of amino acids is higher throughout. The ordinary silage shows a high amino acid formation at the lowest *pH* value, falling thereafter. The figures for ratio of amino acids to volatile bases show that at the lower *pH* values the excess of amino acids is greatest, falling off as the *pH*, and with it the volatile base value, rises. Above 4.0 all three processes are indistinguishable. The reason for the general excellence of A.I.V. fodder is evident from all three curves, but well-made molassed silage does not differ from it between the *pH* 3.5 and 4.5 levels, within which the majority of the A.I.V. fodder and the molassed silage samples fell; 66 and 77 per cent respectively. This is particularly true of the volatile bases, which do not form any appreciable amount of the total nitrogen until the *pH* value is over 4.5. Below this level, the only change which occurs down to *pH* 2.5 is a reduction in the percentage of volatile bases from 9 to 4 per cent, a small change, negligible for most purposes. The somewhat greater formation of amino acids in molassed silage is of little import, since recent work at this Station<sup>(22)</sup> has shown that for milk production this fraction has a value which does not differ markedly from that of true protein.

The silage made from sugar-beet tops does not show any great difference in protein breakdown with or without added acid, the values being relatively low both for volatile bases and amino acids.

### *High-protein silage*

Before leaving this aspect of the changes involved in silage making, it is as well to consider the case of silage made from grass of high crude-protein content. It has proved possible to extract a number of grass silages, which contain over 17 per cent of crude protein in the dry matter, from the individual sample lists. There were six such samples of ordinary silage, ten made with added molasses, six with moderate amounts of mineral acids, and thirty-three of A.I.V. fodder. The *pH* values and

details of the nitrogen content and its constituent fractions are summarized in Table XIII.

Table XIII. *pH, total acidity and protein breakdown in silage of high crude-protein content*

	Ordinary silage	Silage made with added molasses	Silage made with moderate amounts of mineral acid	A.I.V. fodder Mineral acids added in amounts designed to produce a pH of 3.0-4.0
Number of samples	6	10	6	33
Dry matter content (%)	24.0	23.4	24.8	22.7
pH	4.91	4.19	4.32	3.82
Total acidity e.c. N/10 per 100 g. fresh silage	384.3	455.5	400.9	327.9
Crude protein % of dry matter	19.87	17.85	18.65	18.83
"True" protein % of dry matter	8.27	9.07	10.07	12.27
Volatile bases* % of fresh silage	0.82	0.39	0.54	0.26
Volatile bases* % of dry matter	3.42	1.67	2.18	1.15
Amino acids* % of fresh silage	0.99	1.17	1.03	0.75
Amino acids* % of dry matter	4.13	5.00	4.15	3.30
Volatile base N as % of total N	17.21	9.35	11.47	6.11
Amino acid N as % of total N	20.79	28.01	21.84	17.53
Volatile base + amino acid N as % of total N	38.00	37.36	33.31	23.64
"True" protein N as % of total N	41.62	50.81	52.99	65.06
Volatile base + amino acid + true protein N as % of total N	79.62	88.17	86.30	89.70
Ratio $\frac{\text{Amino acids}}{\text{Volatile bases}}$	1 : 1.21	1 : 2.99	1 : 1.90	1 : 2.87

\* Calculated as crude protein, i.e. volatile base or amino acid N  $\times$  6.25.

The ordinary silage group contains two samples, which suffered as a result of seepage of soil water, but these have also been included. The dry-matter content of all four groups did not differ appreciably, that of the A.I.V. fodder being the lowest. The pH values show up the ordinary silage in a bad light, the average value being 4.91. This was not affected unduly by the two bad samples, all six samples showing values well over 4.5. The molassed silages were satisfactory, and only two samples exceeded the average value (4.51 and 5.38), four of them having a pH of under 4.0. The addition of moderate amounts of acid also gave a good silage, as judged from its acidity, only one exceeding pH 4.5. The A.I.V. fodder samples were of satisfactory acidity, seven exceeding pH 4.0, only one of these (4.56) being over 4.34.

The crude-protein contents agree fairly well, the ordinary silage being the highest. The percentage of "true" protein in the crude protein is of the same order as that seen in examining all the samples. The volatile base content shows that the A.I.V. process has controlled the breakdown

Even allowing for the residue of added mineral acids, the amount of lactic acid in the A.I.V. fodder is fairly high, since the figures in Table VI have shown that an appreciable portion of the residual acidity, even in the fodder, is due to lactic acid.

The silages made from sugar-beet tops do not show any differences due to the addition of mineral acids. The potato silage made from steamed potatoes shows a high content of volatile acids and of lactic acid, whilst the sample made with added acid is curious in that it shows no residual acidity. Little can, however, be said of an isolated sample.

In general, the non-volatile acids in good silage are in excess of the volatile acids, as may be seen from the column showing the ratio between these two forms of organic acids. Dox & Neidig<sup>(23)</sup> quote a ratio of 1 : 1.34 in maize silage, as a result of direct estimation of the acids, and Amos & Woodman<sup>(19)</sup> a ratio of 1 : 1.63 for oat and tare silage. The latter, however, add a note of warning showing that in certain cases secondary changes, such as those due to mould action, may reduce the amount of the organic acids to such a low level that the ratio may be misleading, and quote such a sample with a ratio of 1 : 1.33. Judged by this criterion, all the silages with a *pH* of under 4.5 may be classed as good.

In Fig. 2 the average non-volatile and volatile acid contents of the samples of grass silage made by the three main processes have been plotted against *pH*.

The non-volatile acids, residual acidity calculated as lactic acid, are fairly constant in A.I.V. fodder up to *pH* 4.0, after which they fall, and from about *pH* 4.2 onwards are similar to that of ordinary silage. The higher lactic acid content of molassed silage shows up in the curve for this type of silage.

The curves for volatile acids are particularly interesting. Over the range 3.8-4.8 all three types of silage agree very well, but after this the value for ordinary silage rises rapidly, due largely to the formation of butyric acid, as may be seen from Fig. 3. This is not true for molassed silage.

With increasing acidity in the A.I.V. fodder, the volatile acids are reduced still further, though gradually. The ratio non-volatile acids/volatile acids has also been plotted, and show excellent agreement between all three processes above *pH* 4.0.

The reason for the excellence of A.I.V. fodder is apparent in these curves. The acidity of the mass due to residual acidity is constant and high up to *pH* 4.0, and only falls thereafter.

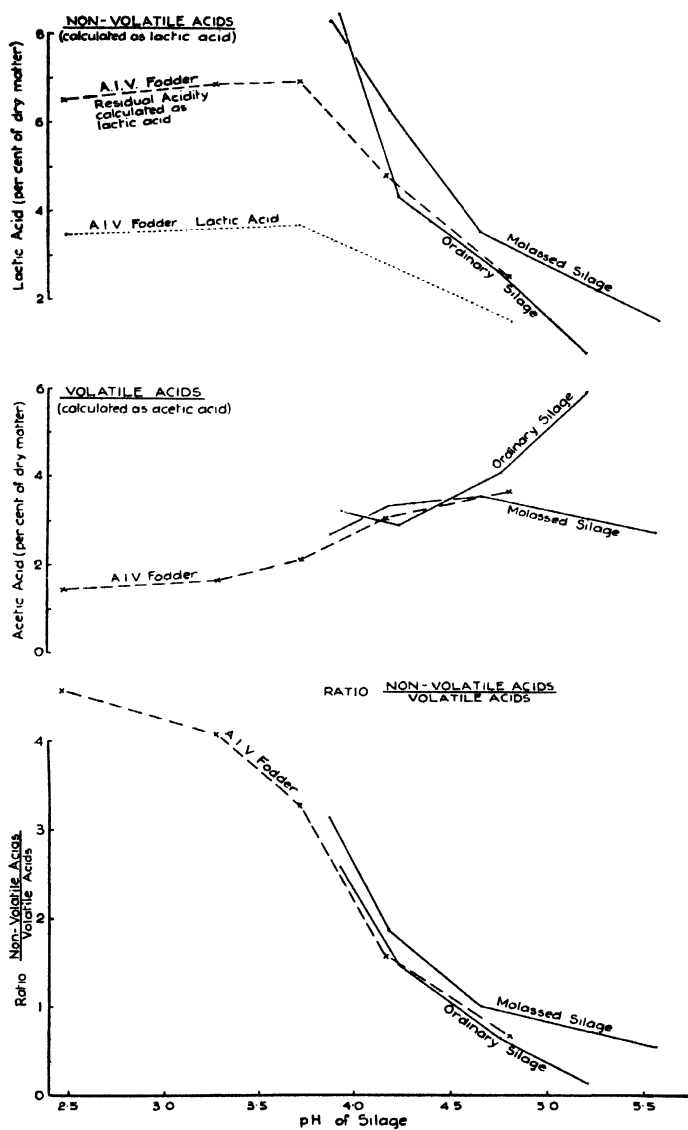


Fig. 2.

The volatile acids rise but slowly with increasing  $pH$  value, and the lower this value the less the chance of butyric acid being present. The volatile acids do not rise any more rapidly where molasses are added, or indeed in ordinary silage until  $pH$  4.8 is reached.

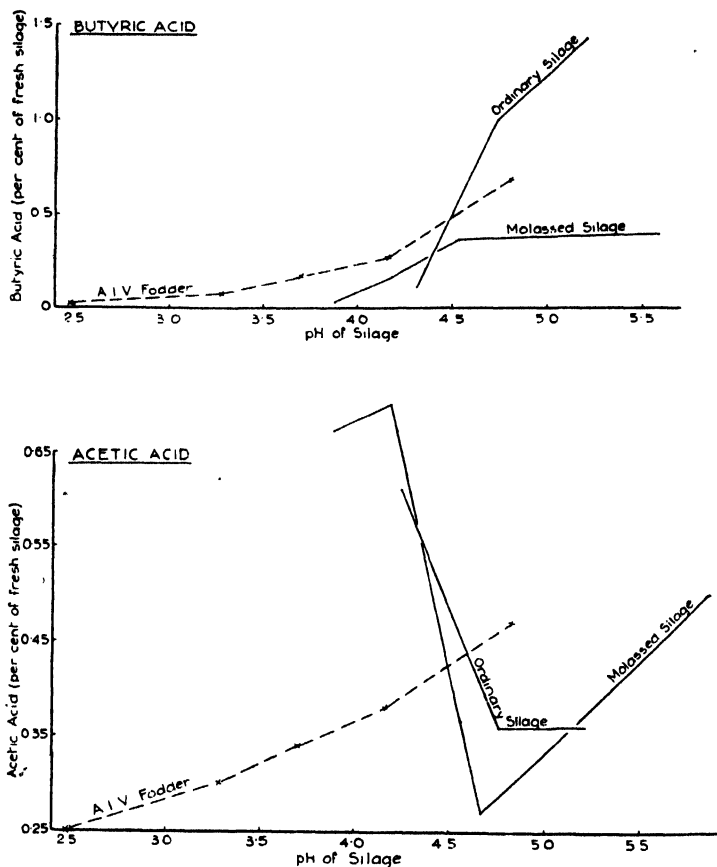


Fig. 3.

The excess of non-volatile acids over volatile acids in A.I.V. fodder falls up to  $pH$  4.0, but at a greater rate thereafter, keeping in line with the other types of silage.

The probable true lactic acid content of the A.I.V. fodder can be calculated from the residual acidity by applying the figures in Table VI, showing the relationship between residual acidity and determined lactic acid. Thus, at a  $pH$  value below 4.0 only 52.4 per cent of the residual

acidity is due to lactic acid, whilst above 4.0 the figure is 60.0 per cent. The curve for probable lactic acid content of A.I.V. fodder has been plotted on this basis, and shows that this type of silage contains less lactic acid than either of the others.

#### ACETIC AND BUTYRIC ACID

It has already been seen that the values for total volatile acids determined by the Foreman method agree well with the results of the Wiegner process. It is, however, important to study the relative formation of butyric acid more closely. The ordinary process of silage making evidently does not succeed in controlling the formation of butyric acid as closely as might be desired, and the amount increases rapidly with the *pH*, reaching a high level between *pH* 4.5 and 5.0, though below this the figure is reasonable. It does show, however, that in the majority of samples appreciable amounts of butyric acid are to be found.

Where molasses is added, a distinct improvement is found at once, even at high *pH* values.

The use of acid as in the A.I.V. process has a beneficial effect also, though it does not seem to possess the advantages over the molasses process which might have been expected. It should be stated that the figures all refer to total butyric acid (free plus combined butyric acid), since this is the only true criterion, as the values for free butyric acid alone may be misleading and show the apparent absence of this compound. Thus, for samples with a *pH* of under 4.0, the molassed silage contained 0.04 per cent of butyric acid (on the fresh silage), whereas the A.I.V. fodder has an average weighted value of 0.13 per cent. Between *pH* 4.0 and 4.5 molassed silage and A.I.V. fodder contain 0.16 and 0.26 per cent butyric acid respectively. These figures show up clearly in Fig. 3, where the values for butyric acid and acetic acid are plotted against *pH*. The control exerted by mineral acid additions on butyric and acetic acid formations can be seen. They both increase with rising *pH*. In ordinary silage and molassed silage, on the other hand, the acetic acid is considerably higher at lower *pH* values, and falls with increasing *pH* values. This is counterbalanced by increases in butyric acid, which give the curve shown in Fig. 2 for the increase in total volatile acids.

Molassed silage compares favourably with A.I.V. fodder in so far as butyric acid formation is concerned, for at the lowest *pH* values this constituent is no higher than in A.I.V. fodder, even when this latter has a *pH* well below 4.0. The critical point of *pH* 4.2 for butyric acid for-



mation is correct in so far as ordinary silage is concerned, for the content rises rapidly thereafter. In A.I.V. fodder and molassed silage it is not so apparent, although below pH 4.2 the amounts of butyric acid are very low, and a large proportion of the samples contain none of it.

Piriaux and his colleagues<sup>(17)</sup> have noted that the formation of butyric acid is not checked at a pH of under 4.2, though at lower values it does appear to be controlled.

From the figures quoted by these workers the values for grass silage have been extracted, and are given below.

Table XV. *Butyric acid content of grass silage (Piriaux et al. (17))*

	No. of samples	Average pH	Butyric acid % fresh silage
Warm fermentation in towers	4	4.1	0.23
Warm fermentation in stacks	3	4.7	0.56
Cold fermentation	3	4.3	0.52
Cold fermentation with added molasses	2	3.9	0.09
Silage made with added acid	6	3.5	0.00
	9	3.9	0.30

These agree satisfactorily with silage made by similar processes and of the same pH value in the present investigation, the value for silage made with added acid of pH 3.9 being less favourable than the present figures, whereas the values for molassed silage agree well, though the number of samples examined by Piriaux was small. It should be mentioned in extenuation that some of the samples given by Piriaux were not made by the A.I.V. process but by allied processes using smaller amounts of acid, but they are indicative.

A great deal of isolated information on the acetic and butyric acid content of silage is available, but the only collection of figures is given by Kuchler<sup>(24)</sup>.

Table XVI. *Butyric acid content of grass silage*

Details	No. of determination	Butyric acid % fresh silage	Author
Normal process: grass uncut	3	1.00	Kuchler <sup>(24)</sup>
Special silos	17	0.10	
Electro silage	13	0.00*	
Addition of bacterial cultures	3	0.03	Kirsch & Hildebrandt <sup>(25)</sup>
Clover, cold fermentation	10	0.52	
Clover, cold fermentation + molasses (1 %)	10	0.00	

\* 1 sample contains 0.07 %.

The figures abstracted from Kuchler's collection of data refer only to silage made from grass, and were compiled before the acid processes

came to the fore. On the whole, they show that the butyric acid content of ordinary silage can be low. Where, however, unchaffed grass was used by the ordinary process, the average value is very high, and no *pH* values are available. The use of special silos, used for the cold fermentation process, results in relatively low values for butyric acid, as does the electro-silage process. The addition of bacterial cultures of lactic acid organisms does not affect the average markedly. The figures quoted by Kirsch & Hildebrandt<sup>(25)</sup> from a series of experiments carried out by the East Prussian Silo Ring on clover silage are included as a matter of interest. The addition of 1 per cent of molasses to a purely leguminous crop—notably a cause of butyric acid fermentation—has had the required result and checked the formation of this acid. A considerable amount of work has been carried out which shows clearly that the formation of butyric acid decreases with the *pH* value, but it is not intended to discuss this here.

The addition of acid to sugar-beet tops appears from the single sample analysed to have reduced the amount of butyric acid formed by half, but it is impossible to discuss results based on single determinations.

### *High-protein silage*

The same criticism can be levelled against the discussion of the results above, as was the case with the protein breakdown; the average crude-protein content is low. The same group of silages, all containing over 17 per cent of crude protein in the dry matter, is summarized below, in so far as the content of volatile and lactic acid is concerned.

It is clear that the ordinary silage process is unsuitable for grass of high-protein content. The amount of lactic acid present is not so high as in any of the other processes, and the volatile acids are relatively high, agreeing closely with the value obtained in Table XIV for silage of similar *pH* value, and this contained large amounts of butyric acid. It is only fair to state that two out of the six ordinary samples were from silos in which some seepage of soil water had occurred, and these gave values of 1.66 and 1.73 per cent of butyric acid. Two other samples, in which this acid was determined, gave values of 0.11 and 0.69. The addition of molasses resulted in a greater amount of lactic acid being formed, and the volatile acids are relatively low and agree well with the values for all the samples (Table XIV). Butyric acid was determined in all the ten samples of molassed silage, and gave an average value of 0.17 per cent. Five of the samples contained no butyric acid, and the only two samples in which the *pH* exceeded the group average (*pH* 4.51

and 5.38) gave values of 0.57 and 0.67 per cent of butyric acid, and account for the greater part of the butyric acid total for the group.

The ratio of volatile to non-volatile acids in the molassed silage was 1 : 2.72, which is satisfactory, whilst in the ordinary silage the volatile acids were in excess. The addition of moderate amounts of acid, with or without molasses, has not been so satisfactory as molasses alone, though distinctly better than the ordinary silage. The three samples examined for butyric acid in this group were those with the highest values for pH, and showed the presence of appreciable amounts of butyric acid (0.67 per cent). Since the other three samples, of lowest pH value, were not examined, it is hardly fair to say more than that butyric acid was present in some of the samples.

Table XVII. *Volatile and non-volatile acid content of grass silage of high-protein content*

	Ordinary silage	Silage made with added molasses	Silage made with moderate additions of mineral acid	A.I.V. fodder Mineral acids added in amount designed to produce a pH of 3.0-4.0
Foreman's method				
No. of samples	6	10	6	33
pH	4.91	4.19	4.32	3.82
Volatile acids % of fresh silage	1.09	0.69	0.88	0.40
Volatile acids % of dry matter	4.54	2.95	3.55	1.76
Residual acidity c.c. N/10 per 100 g. fresh silage	90.4	213.4	136.3	179.1
Residual acidity as lactic acid % fresh silage	0.81	1.88	1.23	1.59
Residual acidity as lactic acid % dry matter	3.38	8.03	4.96	7.00
Ratio $\frac{\text{Non-volatile acids}}{\text{Volatile acids}}$	1 : 0.74	1 : 2.72	1 : 1.40	1 : 3.98
Wiegner's method				
No. of samples	4	10	3	17
pH	4.94	4.19	4.55	3.86
Acetic acid % of fresh silage	0.48	0.56	0.42	0.28
Butyric acid % of fresh silage	1.05	0.17	0.67	0.17

The A.I.V. process here, as with the full data, shows decidedly lower values for volatile acids, and the residual acidity, calculated as lactic acid, is somewhat lower, the ratio of volatile to non-volatile acids (1 : 3.98) being very favourable.

Of the 33 samples some 17 were examined for butyric acid, the average value being 0.17 per cent of the fresh silage. Seven of the samples contained no butyric acid, but three samples (0.49, 0.36 and 0.55 per cent) have been responsible for raising the average.

These results generally confirm the findings of the examination of the whole of the data in all respects, and show that they refer with equal force to silage made from material of high crude-protein content.

### CONCLUSIONS

The conclusions which may be drawn from the examination of the samples of silage are that the use of mineral acids in silage making, according to the A.I.V. process, results in the production of a foodstuff of excellent quality, in which the breakdown of protein and the formation of organic acids, which is so characteristic of ordinary silage, is markedly reduced, though not to the extent which might be expected. The production of volatile bases is reduced to a minimum. The *pH* value of the silage is the most valuable criterion, but must be reinforced by a determination of the amount of volatile bases and volatile acids.

Butyric acid formation is reduced to a minimum, and the lower the *pH* value the greater is the control of this undesirable fermentation. Though the majority of the silages examined have shown a *pH* of the desired level (between 3.0 and 4.0), it is obvious that, in practice, even when the directions are adhered to, a certain percentage of the silages made by the A.I.V. process will show a *pH* reaction above 4.0, but rarely do they exceed 4.5, at which level the quality of the silage is still good. Butyric acid formation is not always controlled, even below *pH* 4.0, but the amount is insignificant. A few samples of silage have been examined in which the use of lower amounts of mineral acid have been used, either alone or with added molasses, but though the number is too small to draw final conclusions, this has apparently had no advantages over the use of molasses alone.

The ordinary process of silage making, as generally applied in practice, where too often due care is not taken during filling, has distinct disadvantages. The formation of volatile bases is much greater than is the case with the A.I.V. process, the volatile acid production is greater and, what is more important, appreciable amounts of butyric acid are often present. This process does not ensure the certainty of producing good silage, which is such an outstanding advantage of the A.I.V. process. The majority of the samples of ordinary silage examined have had a *pH* of over 4.5, though an appreciable number lay between 4.0 and 4.5, and very few showed an acidity of under *pH* 4.0. Where materials of high crude-protein content are ensiled, the disadvantages of the process are accentuated to a marked degree, but for material at an advanced stage of maturity the process is probably quite satisfactory.

In this process, as indeed in all, attention to thorough compaction of the mass and the use of a suitable container are essentials. Too often ordinary silage is spoilt by lack of attention to these points. Care in spreading out the grass is necessary, and the rate of filling must be adjusted to allow the mass to heat throughout to an equal degree. The outstanding feature of this study has been the efficacy of an addition of molasses to the fresh crop during the ensilage process. An addition of about 1 per cent of molasses (15–25 lb. per ton of fresh grass) suitably diluted has resulted in the production of a silage of excellent quality, little inferior in its chemical characteristics to that produced by the A.I.V. process. The *pH* of the mass has been in the neighbourhood of 4.0 in the majority of cases. The breakdown of protein is greater than that obtaining with the A.I.V. process, but this does not proceed too far, and the quantity of volatile bases formed exceeds that of this latter process by a negligible amount. The quantity of amino acids and intermediate more complex nitrogenous compounds formed is greater with the molasses process, but even in the A.I.V. process an appreciable change in this direction is noticeable.

The addition of molasses, as might be expected, increases the amount of lactic acid formed. The quantity of volatile acids is greater than where acid is added, but the control of the butyric acid fermentation is good, and in most samples the amount is negligible. The use of molasses is indicated where silage of high-protein content is made, and will result in the production of a palatable silage of excellent quality. It must again be emphasized that the greatest care must be exercised in tramping the fodder, teasing it out, and allowing time for individual layers, particularly in the bottom of the silo, to heat somewhat. No process that can be devised will be of any value where such precautions are not exercised.

The few samples of silage made with added whey have shown results comparable to those obtained with molasses. Fresh whey, however, cannot be used with such success, as it involves the use of large volumes to ensure the presence of an adequate amount of lactose. Concentrated whey, suitably diluted, is better. It may be said here that in the samples made with added whey, bacterial cultures were added in some cases with the whey, but showed no advantage over whey alone. These experiments have been written up elsewhere(3).

Whilst this study has shown the valuable properties of the A.I.V. and molasses processes in so far as the chemical composition of the product is concerned, it is also essential to know whether the losses of nutrients involved in the two processes differ. Information on this

point has been accumulated at this Station, and will be published in the near future. There is also a gradually accumulating mass of information on this question, the final decision resting on the relative economics of the processes. Nevertheless, it is possible to say that the addition of acids, as in the A.I.V. process, or of molasses, will result in the production of silage of excellent quality and high palatability, if suitable attention is paid to the filling of the silo. In the routine examination of samples of silage the pH should be determined. The Foreman alcohol titration and distillation method, as modified by Woodman, can be applied to the silage extract, and will give all the necessary information about the formation of volatile bases, non-volatile and volatile acids and, together with a knowledge of the crude protein of the sample, will enable an accurate estimate to be made of the value of the silage. In general, it is not necessary further to fractionate the volatile acids, except for special purposes, when the Wiegner method may be used, and will give the absolute amounts of butyric acid present. The results of a series of digestibility trials with silage have been published from this Station (26), which will enable an approximate starch equivalent, protein equivalent and digestible crude-protein value to be applied to the sample, according to its crude-protein content.

#### SUMMARY

A short account is given of the main changes which take place during the ensilage process. Details are given of the methods of analysis used in examining samples of silage for pH, crude-protein, volatile base, amino acid, lactic acid, total volatile acid, acetic acid and butyric acid contents. The results of the examination of 293 samples of silage are discussed. Of these 258 were made from grassland herbage, 24 from silage crops, 9 from sugar-beet tops, and 2 from potatoes. The silages were made in towers, wood-lined pits, and a small number in stacks or clamps. The samples are divided up into those made by the ordinary process, with added molasses, with added whey, with moderate amounts of mineral acid with or without molasses, and with mineral acids added in amounts sufficient to bring the mass rapidly to a pH of 3.0-4.0—the A.I.V. process.

For comparative purposes the silages made by these processes have been divided, within each process, into groups of varying pH reaction.

The greater number of the ordinary grass silages had a pH of 4.5-5.0 (46 per cent). The majority of the remainder (31 per cent) lay between 4.0 and 4.5, and only four samples lay below pH 4.0. With the addition

of molasses better results were obtained, and 47 per cent showed  $pH$  values of 4.0–4.5, and 29 per cent were under  $pH$  4.0. The small number of samples with added whey and moderate additions of mineral acid were also good, average  $pH$  3.86 and 4.32 respectively. The A.I.V. process produced excellent silage, 69 per cent of the samples lying below the desired  $pH$  maximum of 4.0 (45 per cent between 3.5 and 4.0), and 21 per cent had an average value of 4.17. The product from silage crops was similar to the grass silages. The addition of acid to sugar-beet tops resulted in a distinct improvement over silage made with no addition. The formation of volatile bases was greatest in the ordinary silage, but the addition of molasses, or of mineral acid according to the A.I.V. process, reduced this considerably, the degree of base formation being proportional to the acidity of the mass as measured by its  $pH$  value. At the  $pH$  levels at which these two later types of silage overlapped, there was no difference in the efficiency of the processes. The still greater acidity developed in the A.I.V. process reduced the percentage of volatile base nitrogen in the total nitrogen from some 7 down to 4 per cent—a very small difference. The A.I.V. process reduced the formation of amino acids to a minimum, and the molasses process gave the highest proportion of amino acids, which were, however, as with the A.I.V. process, always in considerable excess of the volatile bases. Consideration of silages of high-protein content confirmed the above findings for material with added molasses or mineral acid, but accentuated the disadvantages of the ordinary process. The formation of lactic acid in ordinary silage is proportional to the  $pH$  of the mass, as is also the case with molassed silage, though the values for lactic acid are higher. The lactic acid falls as the  $pH$  rises. The non-volatile acidity in A.I.V. fodder is not all due to lactic acid, but, as might be expected, is relatively high.

The volatile acidity, on the other hand, rises with the  $pH$ , but is kept at a relatively low level in the A.I.V. process. Up to  $pH$  4.8 there is no difference between any of the three processes, but above this value the volatile acid content of ordinary silage rises rapidly, due to an increase in butyric acid. The formation of butyric acid is difficult of control in ordinary silage, more particularly in material of high-protein content. The addition of mineral acids reduces the chances of butyric fermentation markedly, but the molasses process is as efficient in this regard as is the A.I.V. process. There is distinct evidence that butyric acid may be formed in silage at  $pH$  levels below 4.2—the supposedly critical point—but the amounts are relatively low, and it can be accepted in principle that in well-made silage, where the  $pH$  is not in excess of 4.5, the quantity of butyric acid present will be negligible for all general purposes.

In conclusion, it may be stated that though the ordinary process of silage making may be adequate for material at a fairly advanced stage of growth, it is not suitable for high-protein silage, though with due care good quality silage can be made from such material without any addition. To obtain greater certainty of first-class silage, molasses or acid should be added during the filling of the silo, due precautions being taken in the packing of the material. Judged by chemical characteristics, there is no important difference between the two processes, and it is necessary to take into account the relative losses of nutrients and other economic factors before a final decision can be made as to the relative overall efficiency of the molasses and the A.I.V. processes.

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# SEED DISINFECTION

## II. LARGE-SCALE FIELD TRIALS ON THE DISINFECTION OF SEED CORN WITH MERCURY DUST DISINFECTANTS

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### INTRODUCTION

In a previous paper<sup>(1)</sup> it was stated that some mercury dust disinfectants had proved effective in laboratory and small plot tests in controlling certain seed-borne diseases, and that these disinfectants were being further tested in large-scale field trials in East Anglia.

It was stated that although biological laboratory work plays an important part in an investigation of this kind "...the intrinsic test in the determination of fungicidal value is the disease control that is obtained in field experiments". Although small-scale field plots, such as the rod-length units used in the early stages of this investigation, are a valuable supplement to laboratory experiments, it is desirable, before drawing any conclusions as to the efficacy of the treatments, to check the evidence so obtained by making large-scale field trials, using the type of equipment which would be available to the general farmer. It was decided, therefore, to obtain corroborative evidence by arranging a limited number of large-scale field plots, adequately replicated. Trials on the common cereal crops were arranged through the Eastern Counties Provincial Conference, and the counties participating in these experiments were Essex (winter wheat), Cambridgeshire (winter oats), Hertfordshire (spring oats) and Norfolk (spring barley). The writers are indebted to the Agricultural Education staffs of these counties for undertaking the major part of the field work. A detailed description of these field trials is not given but the results obtained are summarized.

### EXPERIMENTAL PROCEDURE

In all cases three main treatments were adopted, viz.:

(1) Seed dusted with an experimental dust containing an organic mercury compound;

(2) Seed from the same bulk as stock (1) but dusted with a proprietary mercury dust; and

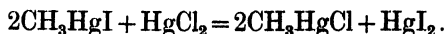
(3) Seed from the same stock as (1) and (2) but not treated in any way against seed-borne diseases.

In the case of wheat, these three treatments were carried out on seed showing no signs of bunt contamination, and also on seed from the same bulk supply but artificially contaminated with a heavy dose of bunt spores. There were thus six different seed treatments used in the wheat trials.

As it was not possible artificially to infect bulk samples of oats and barley with the fungi causing leaf spot of oats (*Helminthosporium avenae*), leaf stripe of barley (*H. gramineum*) and net blotch of barley (*H. teres*), the trial plots were sown with commercial seed. The method of treatment was the same as that previously described, but, in the case of spring oats, the three treatments were made on both of two separate lots of seed of the same variety, the one known to be relatively free from the organism causing leaf spot and the other infected with it. The difference in origin of these two stocks of seed introduced into the trial an additional variant, as the infected stock was apparently more vigorous than the non-infected sample. Comparisons within the two seed types are, however, still valid and provide useful evidence on the matter under investigation.

At three centres the individual plots were approximately one-tenth of an acre in size, each treatment being replicated six times in a randomized block lay-out, but at a fourth centre (the barley trial) the plots were only one-thirty-sixth of an acre in size and each treatment was replicated ten times. The experimental dusts used in these four trials are called A and B and they were made as follows.

For the preparation of experimental dust A, 100 lb. of filler was treated with an aqueous solution containing 0.55 lb. mercuric chloride (equivalent to 0.4 lb. Hg). The solution was diluted sufficiently to secure a uniform distribution during mixing, and the mixture was then dried. The dry product was transferred to a ball mill and 1.375 lb. of methyl mercury iodide added (equivalent to 0.8 lb. Hg). The mixture was dry ground until the reaction was complete, this being according to the following equation:



For the preparation of experimental dust B, 100 lb. of filler was intimately mixed with an aqueous solution containing 1.11 lb. of methyl mercury nitrate (equivalent to 0.8 lb. Hg). The solution was diluted

sufficiently to ensure uniform distribution on the filler during mixing, and the product was then dried. The filler used in these dusts was a non-adsorbent aluminosilicate with an average particle diameter of  $9\mu$ . As stated elsewhere<sup>(1)</sup> it can be shown mathematically that such a filler applied to seed wheat at the rate of 2 oz. per bushel will produce a continuous layer two particles deep.

The wheat and winter oats were treated with dust A and the spring barley and oats with dust B. The proprietary article used was the same in all cases.

#### WHEAT TRIAL

The variety was Victor, drilled on 15 November 1934. Samples of seed, treated and untreated, were sent to the Official Seed Testing Station, Cambridge, for germination tests. These results are recorded in Table I.

Table I. *The percentage germination of wheat treated and untreated*

Treatment	Clean seed	Bunt contaminated seed
No treatment	98	98
Proprietary dust	99	98
Experimental dust A	98	97

In addition to providing evidence on the value of this type of seed disinfectant in controlling bunt, this trial was planned so that quantitative yield data might be obtained. Unfortunately, shortly after the seedlings had speared through, rabbits destroyed large areas of the crop on four of the six blocks and so made it impossible to obtain any reliable data either on plant population or yield of grain. The remaining two blocks of plots also suffered considerable injury, but a strip 20 yd. wide was harvested and an estimate for bunted ears was made on random samples from this produce, approximately 16,000 ears being examined. Though the loss of four blocks has robbed the results of much of their significance,

Table II. *The percentage of bunted ears from treated and untreated seed*

Treatment	Percentage of bunted ears
Clean seed: No treatment	0
Proprietary dust	0
Experimental dust A	0
Contaminated seed: No treatment	11.8
Proprietary dust	0.4
Experimental dust A	0

the bunt estimates shown in Table II afford considerable evidence of the successful control of bunt by the dusts used in this experiment.

No data are presented for plant population or yield of crop at this centre because of the circumstances already described.

The germination tests indicated that there was no obvious phytocidal effect and the figures given in Table II show the effectiveness of the treatments in bunt control.

#### BARLEY TRIAL

The variety Spratt Archer was drilled on 19 March 1935, and estimates of the number of seedlings per treatment were made on two dates, 10 and 22 April. These were based on counts made on twenty-four random foot-lengths of row on each of the ten replications and the data are given in Table III.

Table III. *The mean number of plants per twenty-four foot-lengths of row*

Date	No treatment	Proprietary dust	Experimental dust B	S.E.
10. iv. 35	424.1	458.5	452.3	9.26
22. iv. 35	462.4	489.1	476.3	9.01

Assuming differences equal to three times the standard error to be statistically significant, it will be seen from Table III that both dusts gave a significantly larger plant population at the first count but the differences between dusted and non-dusted plots were not significant at the second count.

Later, on 27 May, estimates of the percentage of leaf stripe and net blotch infection were made for each treatment. These were based on the results of random counts made on each plot. These estimates are recorded in Table IV.

Table IV. *The percentage infection with leaf stripe and net blotch*

Treatment	Percentage of leaf stripe and net blotch
No treatment	5.0
Proprietary dust	1.0
Experimental dust B	0.6
Standard error	0.2

A small amount of wireworm damage occurred at this centre, but the crop did not suffer any serious loss of plant and the plots were harvested

on 10 August. Sheaf weights were recorded and, since these showed no significant differences between treatments, the produce from all replicated plots of the same treatment was bulked and threshed. The yield data are given in Table V.

Table V. *The yield of barley (grain and straw) per acre*

Treatment	Grains (cwt.)	Straw (cwt.)
No treatment	18.03	13.50
Proprietary dust	18.77	15.07
Experimental dust B	17.74	13.27

The small amount of the leaf-stripe disease had apparently no significant effect on the yield of barley at this centre, and it was not possible to detect any beneficial effect on yield from the slightly higher plant populations shown by the treated seed at the first seedling count. This suggests that any apparent "stimulating" effect in the early stages of growth does not necessarily result in an increased yield.

#### WINTER OAT TRIAL

The variety Grey Winter was drilled on 12 October 1934. The seed was from the farmer's own stock and was not artificially contaminated with the spores of any seed-borne disease. Samples of seed, treated and untreated, were sent to the Official Seed Testing Station for germination tests. These are recorded in Table VI.

Table VI. *The percentage germination of winter oats treated and untreated*

Treatment	Percentage germination
No treatment	99
Proprietary dust	99
Experimental dust A	98

Germination and early growth were satisfactory on all plots, but foot-length counts showed wide differences in plant establishment between different treatments. Twenty random foot-lengths of row were pegged out on each plot and the numbers of seedlings in these lengths were counted on 29 October, and again on 27 November 1934. A further examination at the commencement of tillering showed a slight reduction in the number of seedlings on several foot-lengths, so that the November count probably represents the final germination figures. The results from these two counts are given in Table VII.

Table VII. *The average number of seedlings per twenty foot-lengths of row*

Date	No treatment	Proprietary dust	Experimental dust A	S.E.
29. x. 34	438	433	510	26
27. xi. 34	494	497	578	24

The differences between treatments just failed to reach statistical significance at the first count but, at the second count, the plots from seed treated with the experimental dust A had a significantly higher seedling population than those from the untreated seed or the seed treated with the proprietary dust. It is interesting to note that, despite the apparently larger seedling population on the experimental dust A plots at the first count, these plots increased in lead very slightly by the time the second count was made. At this centre, therefore, the evidence suggested that the experimental dust A did not only increase the *rate* of germination but caused an increase in the total number of seedlings which succeeded in reaching the surface of the ground.

Several speculative suggestions may be put forward to account for this increase and some may be examined briefly.

(i) The increased plant establishment was caused by the decrease in mortality by pre-emergence blight (*Helminthosporium avenae*). There was, however, very little leaf spot showing in any of the plots, and it is unlikely that there was any heavy mortality from pre-emergence blight, since the seedlings which pierced through the soil did not show an appreciable leaf spot attack.

(ii) Infection by loose smut (*Ustilago avenae*) may have killed some of the seedlings, for subsequently it was found that this disease was very severe on some of the plots. Some evidence to support this suggestion is given by Mr S. Dickinson, School of Agriculture, Cambridge, who states to one of the writers *in litt.*: "Referring to our conversation yesterday on the possibility of seedlings being killed by smut fungi, my observations were made in 1929 using Grey Winter as a host plant. I was then germinating and infecting my seedlings in the laboratory and planting them out after about seven days. A high percentage of distortion led me to make some further experiments which showed conclusively that oat and barley seedlings could be malformed, and later killed, by the attack of their appropriate smuts."

(iii) The dust treatment may have protected the seed against attack by disease organisms in the soil.

Observations made on 15 November 1934 showed that very few

plants were affected by leaf spot, even on the plots grown from the untreated seed; and, consequently, detailed estimates to determine the effectiveness of the treatments in controlling leaf-spot disease were not made. Later in the season, however, it was noticed that there was a considerable amount of loose smut in the oat crops in the district in which this farm was located and a survey of the experimental plots, made on 5 July 1935, showed marked differences in the amount of smut present. An estimate of the intensity of attack was made by examining 500 panicles taken at random from each plot, i.e. 3000 from each treatment, and the percentage infection with loose smut based on this estimate is shown in Table VIII.

Table VIII. *The percentage of smutted panicles from treated and untreated seed*

Treatment	Percentage of loose smut
No treatment	34.5
Proprietary dust	15.9
Experimental dust A	0.03
Standard error	1.05

It is interesting to note that one type of dust effected almost complete control whilst the other reduced it by about one-half. The observation supports a suggestion previously made "...that although some of the organic mercurials are specifics for certain seed-borne diseases, it does not necessarily follow that they are a panacea for all of them" (1).

#### SPRING OATS TRIAL

Two stocks of seed, both of the variety Marvellous, were drilled on 8 March 1935,  $7\frac{1}{2}$  weeks after they had been treated with the dusts. One stock of seed was relatively free from infection by *Helminthosporium avenae* (referred to later as "clean seed") and the other was badly infected (referred to later as "infected seed"). Samples of the seed,

Table IX. *The percentage germination of spring oats treated and untreated*

Seed	Treatment	Percentage germination
Clean	No treatment	90
"	Proprietary dust	86
"	Experimental dust B	83
Infected	No treatment	96
"	Proprietary dust	97
"	Experimental dust B	98



treated and untreated, were sent to the Official Seed Testing Station for germination tests and the results of these are given in Table IX.

It will be seen from Table IX that the infected seed had a uniformly higher percentage germination than the clean seed. This should be remembered when considering the field data, for the effects of this difference are clearly seen throughout the trial. Seedling population counts were made at this centre on twenty random foot-lengths of row on each plot, on 11 April and again on 29 April, and the percentage infection with *Helminthosporium avenae* was estimated on 24 April. These results are given in Table X.

Table X. *The average number of seedlings per twenty foot-lengths of row and the percentage infected with Helminthosporium avenae*

Seed	Treatment	Total seedlings		Percentage infection with <i>H. avenae</i> 24. iv. 35
		11. iv. 35	29. iv. 35	
Clean	No treatment	152	131	4.0
"	Proprietary dust	220	202	0.2
"	Experimental dust B	254	227	0.2
Infected	No treatment	214	200	22.0
"	Proprietary dust	252	225	0.5
"	Experimental dust B	300	269	0.2
	Standard error	26	22	1.2

It will be seen from Table X that, with both types of seed, the dust treatments led to an increased seedling population, though the increase was only statistically significant in the case of experimental dust B. Furthermore, both types of dust proved effective in controlling leaf spot, as shown by the estimates made on 24 April.

Unfortunately, between the first and second counts the crop suffered considerably from wireworm attack and, at the final count, lower seedling numbers were found on every treatment. Although the extent of the reduction was very similar on all treatments, the effect of experimental dust B on seedling population was still significant. The severity of the wireworm attack was estimated by Dr I. Thomas, The School of Agriculture, Cambridge, on 21 May, who assessed the average loss of plant over the whole trial area at 39 per cent.

The produce from each plot was harvested and threshed separately and the yields of grain from each treatment are given in Table XI.

Although the figures in Table XI show appreciably heavier yields from seed treated with protective dusts, the experimental error was unusually high, due, probably, to the irregularity of plant establishment

caused by the wireworm attack. The yield differences were not statistically significant.

Table XI. *The yield of oats (cwt. per acre) from treated and untreated seed*

Seed	Treatment	Yield of grain (cwt. per acre)
Clean	No treatment	13.1
"	Proprietary dust	15.2
"	Experimental dust B	17.5
Infected	No treatment	16.7
"	Proprietary dust	17.9
"	Experimental dust B	19.6

#### TOXICITY, VESICANT ACTION AND OTHER PROPERTIES OF EXPERIMENTAL DUSTS A AND B

These dusts are poisonous and the toxicities to mice of the pure mercury salts used in them are 1.7 times those of mercuric chloride. They have a penetrating and disagreeable odour and they are vesicant. These properties have been noted in a previous paper<sup>(1)</sup>. If dusts of this type are used for experimentation, the above facts must be remembered, adequate care observed, and efficient precautions taken to prevent the dust from coming into contact with the skin, or being absorbed in any way into the system.

#### SUMMARY

1. Treatment in bulk of the grain of wheat, barley, winter oats and spring oats with a proprietary dust, and two experimental dusts A and B which contained organic compounds of mercury, had no harmful effect on germination when drilling followed shortly after treatment. In the case of spring oats, drilling was delayed  $7\frac{1}{2}$  weeks after treatment without any harmful effects on germination.

2. The proprietary and experimental dusts both proved effective in controlling bunt disease of wheat, and the leaf-stripe and net-blotch diseases of barley.

3. In the case of barley, the proprietary and experimental dusts both increased the speed of "brairding" but not the final plant population. This hastening of seedling growth did not lead to a higher yield.

4. In the case of winter oats, only the experimental dust A gave a significant increase in plant population and a satisfactory control of the loose smut disease.

5. Both the proprietary dust, and the experimental dust B gave a significant increase in plant population in spring oats, and both gave a satisfactory control of the leaf-spot disease.

6. The results suggest that different organic mercury compounds do not affect the speed of "brairding" of all cereals to the same extent; and a dust which controls certain seed-borne diseases, such as leaf spot of oats and bunt of wheat, will not necessarily control all seed-borne diseases, e.g. loose smut of oats. In selecting a disinfectant dust it is therefore desirable to know what forms of seed-borne disease it will control.

7. The poisonous, vesicant and other properties of the experimental dusts A and B are noted and it is stated that efficient precautions must be taken if they are used for experimentation.

#### REFERENCE

- (1) DILLON WESTON, W. A. R. & BOOER, J. R. *J. agric. Sci.* (1935), **25**, 628.

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## SEED DISINFECTION

### III. EXPERIMENTS ON THE GERMINATION OF PEAS. SEED PROTECTION BY THE USE OF DISINFECTANT DUSTS CONTAINING MERCURY

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(With Plate I)

#### INTRODUCTION

IN a previous paper<sup>(1)</sup> reference was made to the subject of seed disinfection, in particular to an investigation on disinfectant dusts containing mercury. It was shown that by the use of certain of these dusts the common seed-borne diseases of cereals (excluding the loose smuts of barley and wheat) could be controlled. The present observations record further work, chiefly greenhouse and field studies, that has been done on green peas. This crop would appear to be of increasing economic importance, since in the past ten years the acreage devoted to it has increased by approximately 49 per cent. Its successful cultivation is often much influenced by climatic and soil conditions, particularly during the first few weeks after sowing. If adverse weather conditions follow, poor germination may result although the seed sown may have been viable and not necessarily diseased. Failures of such a type are usually associated with the rotting of the ungerminated seed in the soil, but from the same bulks, samples sown under more favourable conditions may germinate well and produce a satisfactory crop. Ogilvie<sup>(2,3,4)</sup> finds that in the Western Advisory province *Ascochyta Pisi* and *Mycosphaerella pinodes* are two causes of the early failure of pea plants, and that "pea sickness" is associated with a strain of the eelworm *Heterodera schachtii* and with foot-rot caused by various species of *Fusarium*. Premature dying-off of the plants is accompanied sometimes with *Heterodera schachtii* and foot-rot and sometimes with foot-rot alone. In

the Eastern Advisory province we have not as yet recorded *Heterodera* on peas, but *Fusaria* have been cultured from plants dying prematurely in the field and also from seed rotting in the soil, and *Ascochyta Pisi* has been associated with weak development of seedlings.<sup>1</sup> It is not our purpose here, however, to discuss the relative pathogenicity of the organisms causing poor plant establishment, the symptom complex of such failures, or the factors responsible for the rotting of the seed in the soil, but to indicate how, in certain circumstances, better stands may be established and rotting of the seed decreased. In this connexion the following laboratory experiment is of some interest.

Four large Petri dishes (diam. 9 in.), A, B, C, D, containing sterilized potato agar were prepared, and two of the plates, A and B, were lightly contaminated with soil that had not previously grown peas. Fifty seeds of the variety Daisy were then placed on the medium in each plate. The seeds in plates A and C were previously dusted with a mercury dust disinfectant that we had prepared ( $P_5$ ), but the seeds in plates B and D were not dusted. Nine days later the plates were examined, the observations being recorded in Table I.

Table I. *The percentage germination, and percentage of seeds and seedlings showing fungus growths, recorded on treated and untreated pea seed*

Plate No.	Treatment of Petri plate	Treatment of seed	Percentage germination	Percentage of seeds or seedlings showing mould growth	Amount of mould contamination on medium
A	Sterilized medium contaminated with soil	Dusted seed	88	8	Very slight
B	„	Undusted seed	64	54	Completely contaminated
C	Sterilized medium	Dusted seed	94	10	No contamination
D	„	Undusted seed	94	18	Severe contamination

There is some evidence here not only of seed protection but also of local sterilization in the vicinity of the seed.

When untreated pea seeds are plated out on sterile media the mould spores and mycelia on or in the testa very quickly germinate or reawaken into activity and soon cover seeds and media and exert an inhibiting effect

<sup>1</sup> After this paper was written, Mr F. R. Petherbridge recorded from Suffolk the failure of a pea crop due to *Heterodera* (*Ministry of Agriculture Monthly Summary of Plant Pests and Diseases in England and Wales*, No. 7, 11 August 1936).

on germination. Brown lesions often appear on the radicles when these come into contact with the mould or bacterial colonies, in many cases the cotyledons decay, and a high mortality may result. If, however, the seed is disinfected with a fungicidal dust that is permanent and gives a good cover, much of this decay is prevented. This aspect is illustrated in Pl. I which shows the result of plating out on sterile media dusted and undusted seed.

A further illustration is given by an experiment that was made on the germination of sweet pea seed. A new variety X was received from a well-known specialist on these seeds who complained that the variety was inclined to germinate badly and to rot in the soil. The seeds when examined by the unaided eye did not show any obvious disease symptoms. When they were plated out on sterile potato agar contained in large Petri dishes they germinated well, but a copious mould growth consisting chiefly of *Penicillium* developed.

Equal quantities of five different samples of this seed which had been grown either in British Columbia or England were then sown in five boxes in the laboratory in soil that had not previously grown peas. It should be added that the soil was heavy in character, was over-watered and had been well packed down in the boxes, producing conditions somewhat unfavourable to germination. In each box half of the seeds were treated with a mercury dust disinfectant that we had prepared ( $P_4$ ) and half were left untreated. The total percentage germination of the untreated samples was 5.6, and in the different boxes the variation was from 0 to 12. The total percentage germination of the treated seed was 84, and the variation in the boxes from 76 to 96. Later the untreated seed was sifted from the soil and it was found to be rotting, being bacterial, mouldy and infested with saprophytic eelworm. A *Fusarium* sp. was isolated from some of this rotting seed.

These and similar observations indicated that a protective fungicidal covering applied to these seeds might in field practice assist in resisting the effects of soil organisms or in alleviating the conditions resulting in the rotting of viable seed and so help to establish a satisfactory plant in those cases where viability, as judged by the standard laboratory method, was satisfactory, but where field conditions imposed handicaps sufficiently severe to result in relatively low field germination. At the same time it was thought that the application of such dusts to the seed might tend to reduce the effects of seed-borne parasitic fungi. Ogilvie<sup>(2)</sup>, p. 116) in 1932 noted the peculiar effect of a proprietary mercury dust disinfectant in preserving the cotyledons in a firm state for a long time.

## SMALL-SCALE GREENHOUSE AND FIELD TESTS

A number of preliminary greenhouse tests were made, the most promising dusts were selected and the following experiments were carried out to establish what correlation could be obtained between trials in the greenhouse and in the field.

*Experiment 1*

Varieties: Witham Wonder and Senator, both 1932 crop and known to be infected with *Ascochyta*.

Laboratory germination: Witham Wonder, 72 per cent; Senator, 64 per cent.

Dusting method: Seed with an excess of dust shaken for 1 min. in

Table II. *The results obtained in Exp. 1*

Treatment	Greenhouse experiments				Field experiments		
	Percentage germination after 2 weeks	Total percentage germination after 5 weeks	Diseased plants as percentage of total produced*	Percentage germination after 4 weeks	Total percentage germination after 7 weeks	Percentage of total plants with diseased stems, cotyledons or roots	Weight of total plants in grammes
			Witham	Wonder			
P <sub>1</sub>	5	38	13	29	30	56	93
P <sub>2</sub>	7	37	24	42	43	14	125
P <sub>3</sub>	10	45	13	53	55	17	174
P <sub>4</sub>	18	63	19	69	71	1.4	249
P <sub>5</sub>	18	76	15	68	74	0.7	292
P <sub>6</sub>	11	76	7	74	78	0.6	301
P <sub>7</sub>	14	52	9	54	54	16	174
P <sub>8</sub>	16	48	16	50	50	9	157
P <sub>9</sub>	19	45	21	65	65	8	220
Untreated:							
A	11	37	12	30	31	39	102
B	9	38	23	39	40	30	118
C	9	39	27	45	47	22	136
D	5	37	27	—	—	—	—
				Senator			
P <sub>1</sub>	12	34	18	28	29	26	74
P <sub>2</sub>	14	35	15	36	35	74	90
P <sub>3</sub>	20	41	13	30	30	53	75
P <sub>4</sub>	16	47	10	56	58	23	173
P <sub>5</sub>	21	66	11	69	73	0.7	327
P <sub>6</sub>	21	69	8	63	75	0.7	298
P <sub>7</sub>	24	56	11	51	54	18	246
P <sub>8</sub>	27	53	9	35	39	17	128
P <sub>9</sub>	26	53	11	27	29	17	108
Untreated:							
A	22	49	24	25	26	35	55
B	15	47	26	24	25	26	78
C	18	39	23	21	23	24	53
D	10	29	26	—	—	—	—

\* Chiefly plants with *Ascochyta* lesions on stems. The composition of these dusts is given later in the text. P<sub>8</sub> and P<sub>9</sub> are proprietary dusts.

a stoppered glass jar. Excess dust then removed by sifting for 30 sec. on a 1 mm. sieve.

Date of sowing: 26 and 27 March 1934.

Greenhouse technique: Unsterilized potting soil in boxes, seed planted 1 in. deep, fifty seeds per box, four replicates per treatment; sixteen replicates of untreated seed as control. After 5 weeks seedlings dug up, counted and diseased plants recorded.

Field technique: Seed planted by hand  $1\frac{1}{2}$  in. deep at 2 in. intervals. Four rows of fifty seeds each, three four-row lots of untreated seed as control. After 7 weeks seedlings taken up, weighed and the percentage of disease recorded.

The results of these experiments—shown in Table II—suggested that the three treatments  $P_4$ ,  $P_5$  and  $P_6$ , held some promise as seed protectives when applied to peas of poor quality, and it will be noted that reasonably good agreement between the greenhouse and field tests was obtained. Ogilvie (3), p. 119) has recorded that seed treated with a proprietary disinfectant containing mercury gave a crop increase of 7.6 per cent.

### Experiment 2

Seed used: Two different bulks of mixed seed, one of dwarf varieties, the other of medium height.

Dusting method: As in Exp. 1.

Date of sowing: 12 and 13 April 1934. Field tests only were made.

Technique: 300 seeds for each treatment. Five controls of 300 seeds for medium and three for the dwarf variety. Counts made 6 weeks after sowing, when plants showing diseased stems or roots were counted and the number of plants with rotted cotyledons determined.

Table III. *The percentage germination and disease in two different bulks of garden peas after the seed had been treated with dusts containing varying percentages of methyl mercury phosphate*

Treatment	Medium variety			Dwarf variety		
	Percentage plant establishment	Percentage of plants showing diseased stems or roots	Percentage of plants with rotted cotyledons	Percentage plant establishment	Percentage of plants showing diseased stems or roots	Percentage of plants with rotted cotyledons
$P_4$	72	23	15	72	49	52
$P_5$	69	38	29	80	63	41
$P_6$	67	40	30	70	55	30
Untreated:						
A	46	49	58	59	71	80
B	50	40	84	56	75	73
C	57	62	80	56	73	76
D	47	55	80	—	—	—
E	54	66	87	—	—	—



The results as they apply to  $P_4$ ,  $P_5$  and  $P_6$  and the untreated plots are given in Table III, which shows the percentage germination and disease in two different bulks of garden peas after the seed had been treated with dusts containing varying percentages of methyl mercury phosphate.

This trial shows an increase in plant establishment with the treated seed and also shows that there is less root and stem rot and rotting of the cotyledons. It may indicate that the effect of such fungicidal dusts is to protect the cotyledons from the depredations of soil organisms and so maintain a relatively high proportion of the food materials directly available for the use of the developing plantlet. An inference which might be drawn from this trial is that the effect of such powders is one of "stimulation". It may be stated, however, that controlled experiments in sterile media with a number of different seed species have never in the writers' experience shown any significant stimulation, and they are of the opinion that apparent stimulating effects are due entirely to the protection afforded to the seed and its food reserves.

### *Experiment 3*

The results of this experiment were of interest because of certain adverse effects which were noted.

Variety: First of All.

Laboratory germination: 63 per cent.

Dusting method: As in Exps. 1 and 2.

Date of sowing: 9 and 10 May 1934.

Greenhouse technique: As in Exp. 1, using 1000 seeds per treatment, and control.

Field technique: As in Exp. 1, using 1000 seeds per treatment and control in rows of 100 seeds each.

The greenhouse tests were kept well watered, but the field tests suffered from drought. The results indicated an increased plant establishment in the greenhouse tests but not in the field; in both series the rotting of cotyledons was reduced. In some of these plots dwarfed plants were produced indicating that an excessive amount of dust had been applied. The symptoms of this phytocidal effect have been given elsewhere<sup>(1)</sup>.

Exp. 3 served to emphasise the fact that hitherto the amount of dust applied was not under strict control. Exps. 4 and 5 were therefore made, in order to study the influence of the rate of application of the dust and the effect of varying the percentage of the mercury compound.

*Experiment 4*

This was similar to Exp. 3, but the seeds were dusted at the rate of 2 oz. per bushel. Greenhouse tests only were made and the seeds were sown on 4 June 1934. There was an increase in the percentage of plants produced as compared with the control and evidence also of cotyledon protection, but the differences were not so marked as with the earlier sown trials.

*Experiment 5*

The bulk of seed used was the same as in the two previous trials and dusts containing 2, 3 and 4 per cent Hg as methyl mercury phosphate or nitrate were applied at the rates of 2, 4 and 5 oz. per bushel. No field trials were made.

Date of sowing: 14 August 1934.

Greenhouse technique: 200 seeds per treatment in boxes containing fifty seeds each. Controls: eighteen boxes each containing fifty seeds. Counts made 3 weeks after planting.

The results that apply to Exp. 5 are given in Table IV, which shows the percentage germination and disease present in the variety First of

Table IV. *The percentage germination and disease present in the variety First of All after the seed had been treated with varying concentrations and variable loads of two methyl mercury salts*

Treatment	Percentage germination after 7 days	Total percentage of plants produced	Percentage of plants with rotted cotyledons	Plants with rotted coty- ledons as percentage of total plants produced
A = M.M.P. 2% at 2 oz. per bushel	15	16	9	56
B = M.M.P. 2% at 4 " "	19	21	17	81
C = M.M.P. 2% at 5 " "	24	23	17	73
D = M.M.P. 3% at 2 " "	18	21	13	62
E = M.M.P. 3% at 4 " "	25	26	16	62
F = M.M.P. 3% at 5 " "	41	42	16	38
G = M.M.P. 4% at 2 " "	23	23	11	48
H = M.M.P. 4% at 4 " "	38	37	17	46
J = M.M.P. 4% at 5 " "	51	51	18	35
K = M.M.N. 2% at 2 " "	14	17	9	53
L = M.M.N. 2% at 4 " "	18	20	17	85
M = M.M.N. 2% at 5 " "	25	29	17	59
N = M.M.N. 3% at 2 " "	22	25	16	64
O = M.M.N. 3% at 4 " "	24	31	20	65
P = M.M.N. 3% at 5 " "	37	41	18	44
Q = M.M.N. 4% at 2 " "	19	22	14	64
R = M.M.N. 4% at 4 " "	26	26	16	62
S = M.M.N. 4% at 5 " "	44	46	17	37
Untreated = average of 18 boxes	9	12	9	75

*Note.* The percentages in the first column refer to the percentage of mercury present as methyl mercury phosphate (M.M.P.) or methyl mercury nitrate (M.M.N.).

All after the seed had been treated with varying concentrations and variable loads of two methyl mercury salts.

Compared with the controls the treatments showed an increased percentage of plants produced and there appeared to be a fairly regular increase as between treatment and treatment, corresponding to the increased concentration and rate of application. The protective effect of these dusts, as judged by the percentage of plants with rotted cotyledons was again shown.

#### DISCUSSION OF PRELIMINARY GREENHOUSE AND FIELD TESTS

The evidence from these and other tests that were made suggested that, under the conditions imposed, the dusting of peas with certain organic mercury compounds gave some promise of ensuring an increased plant establishment, and it seemed that one of the important effects of these dusts was in the protection that they afforded to the cotyledons. It was inferred that this protection not only prevented a gangrenous condition of a vital portion of the seed but also caused some local disinfection of the soil in the neighbourhood of the seed and so, under adverse growing conditions, conserved its food supplies. This effect appeared to be more pronounced where conditions were less favourable, as with earlier sowings, suggesting that the date of sowing would influence the response to such treatments.

As this preliminary work seemed promising a number of large-scale field trials were carried out. These were started in the autumn of 1934 and continued in the late spring and early summer of 1935.

#### *Field Experiment 1*

A stock of Laxton's Superb, laboratory germination 76 per cent, was obtained; it was expected that any advantages to be gained from the dusting of peas with organic mercury compounds would probably be more marked on bulks of only medium quality. A third of the amount of seed required to sow the total area available was dusted at 2 oz. per bushel with a proprietary compound, a third at 3 oz. per bushel with the experimental dust P<sub>5</sub>, and the remainder left undusted for control purposes.

Sowing took place on 7 November 1934, in a heavy clay overlying gault. The area was divided into three blocks and each of these contained nine plots each of one drill width, 80 ft. long and randomized over the area sown. The drill was set to sow seven rows, 1 ft. apart, leaving 3 ft. between plots. Owing to the feeds choking similar seed rates were not

obtained. The treated seed was sown at 4.6 bushels per acre, and the controls at 4 bushels.

By 30 November, seedlings were showing through and the first germination count was made on 11 December—5 weeks after sowing. In making this count, three 1 yd. lengths were taken at random in each row of each plot and the number of seedlings counted per yard run. These results are summarized in Table V.

Table V. *The plant establishment obtained when bulks of the variety Laxton's Superb were treated prior to sowing with certain seed disinfectants*

Treatment*	Total No. of plants in 3 × 7 × 9 yd.	Values when C = 100	Average No. of plants per yard run	Average No. of seeds planted per yard, estimated	Plants produced as percentage of seed sown
A	3090	149.56	16	34	48.09
P <sub>5</sub>	3271	158.32	17	33	52.45
Control	2066	100.00	11	29	37.69

\* A = proprietary article. P<sub>5</sub> = 2 per cent Hg as methyl mercury phosphate incorporated with china clay.

The figures in the value column in Table V are not strictly comparable because the rates of sowing were somewhat different. A more faithful comparison is obtained when the values of the counts are worked out on the basis of the known seeding rate. In that case the comparable figures are as follows:

$$\begin{aligned} A &= 127.6 \\ P_5 &= 139.2 \\ \text{Control} &= 100.0 \end{aligned}$$

A statistical analysis<sup>1</sup> of the complete figures shows that for the treatments A and P<sub>5</sub>, the figures are significantly greater than the control, and that the difference between A and P<sub>5</sub> just escapes significance at 5 per cent. If the control is ignored and the difference between A and P<sub>5</sub> only considered, this is shown to be significant at 2 per cent.

*Yield of pods.* Between 24 and 28 June 1935, all pods in a fit state for marketing were picked and the weight of pods from each plot recorded. The total yields per treatment were as follows:

Treatment	Yield in lb., total	Values when C = 100
A	594	132.3
P <sub>5</sub>	700	155.9
Control	449	100.0

<sup>1</sup> The results obtained from the various field experiments were examined statistically by Mr B. Brandreth and we gratefully acknowledge his assistance.

A statistical analysis of the figures obtained showed that  $P_5 > A > C$ , and that the differences are highly significant in each case. The differences in these plots were noted from the time the seed sowed and the control plots could be picked out at a distance.

### *Field Experiment 2*

A bulk of the variety Little Marvel, laboratory germination 99 per cent, was used. The bulk was treated as in the previous trial but dusting was at the rate of 2 oz. per bushel in both cases. The area available allowed of twelve plots of one drill width, for both treatments and the control. The plots were randomized and sown on 13 March 1935, in a similar soil to the previous experiment. Each plot was 81 ft. long. The rate of sowing was approximately 2.8 bushels per acre throughout. Seedlings were showing by 2 April and well through on 11 April. A germination count was made on 23 April, 6 weeks after sowing, when a strip 4 yd. wide was marked off through the centre of all plots and the number of seedlings counted per 4 yd. run in each row of each plot. The results of this trial are given in Table VI.

Table VI. *The plant establishment obtained when bulks of the variety Little Marvel were treated prior to sowing with certain seed disinfectants*

Treatment	Total No. of plants in 12 × 7 × 4 yd.	Values when C = 100	Average No. of plants per yard run	Average No. of seeds planted per yard, estimated	Plants produced as percentage of seed sown
A	8499	106.3	25.3	36	70.3
$P_5$	8391	104.9	24.9	36	69.1
Control	7994	100.0	23.8	36	66.1

A statistical analysis showed that the difference between A and the control just escapes significance at 5 per cent. The yield of pods was not estimated.

### *Field Experiment 3*

A stock of Thomas Laxton peas, laboratory germination 97 per cent, was obtained and treated as in the previous experiment. Twelve plots, each 75 ft. long and of one drill width for each treatment and the control, were randomized throughout the area to be sown, drilling taking place on 2 April 1935, at the rate of approximately 3.1 bushels per acre. The soil was similar in character to that of the previous experiments. Seedlings were well through in all plots by 29 April and the germination count was made on 7 May, 5 weeks after sowing, in a manner similar to that previously described. The figures from this count are given in Table VII.

Table VII. *The plant establishment obtained when bulks of the variety Thomas Laxton were treated prior to sowing with certain seed disinfectants*

Treatment	Total No. of plants in 12 × 7 × 4 yd.	Values when C = 100	Average No. of plants per yard run	Average No. of seeds planted per yard, estimated	Plants produced as percentage of seed sown
A	6050	106.98	18.0	28	64.3
P <sub>5</sub>	5656	100.02	16.8	28	60.1
Control	5655	100.00	16.8	28	60.1

*Yield of pods.* Pods in a state fit for marketing were picked between 5 and 11 July 1935, from twenty-four plots, i.e. eight replications of A, P<sub>5</sub> and the control. The total yields were as follows:

A = 710 lb.	Values when C = 100	A = 113.05
P <sub>5</sub> = 714 lb.		P <sub>5</sub> = 113.7
Control = 628 lb.		Control = 100.0

The difference between the treatments and the control is not statistically significant at 5 per cent.

#### *Field Experiment 4*

The variety Daisy, laboratory germination 86 per cent, was used. Elsewhere it was said to have given rather poor results in the field and, although it was very late in the season, a trial on the lines of the previous one was made. After dusting two portions, as in the other field trials (at 2 oz. per bushel of seed), twelve replications of the two different treatments and the control were sown on 4 June 1935, the plots being of one drill width and 54 ft. long. The rate of sowing was approximately 3 bushels per acre. A germination count was made on 24 June, 3 weeks after sowing, the procedure being similar to that previously described. The results are given in Table VIII.

Table VIII. *The plant establishment obtained when bulks of the variety Daisy were treated prior to sowing with certain seed disinfectants*

Treatment	Total No. of plants in 4 × 7 × 12 yd.	Values when C = 100	Average No. of plants per yard run	Average No. of seeds planted per yard run	Plants produced as percentage of seed sown
A	7585	105.6	22.6	30	75.9
P <sub>5</sub>	7296	101.6	21.7	30	73.07
Control	7178	100.0	21.3	30	71.8

The differences between the treatments and the control are not statistically significant. No figures are available for yield of pods.

A tabular comparison of the four field trials, based upon the number of plants produced, expressed as a percentage of the seed sown, is given in Table IX.

Table IX. *The number of plants produced expressed as a percentage of the seed sown*

Treatment	Laxton's Superb	Little Marvel	Thomas Laxton	Daisy
A	48.09	70.3	64.3	75.9
P <sub>5</sub>	52.45	69.1	60.1	73.1
Control	37.69	66.1	60.1	71.8

The relative values of A and P<sub>5</sub>, from the above figures, when the control equals 100, are as follows:

Treatment	Laxton's Superb	Little Marvel	Thomas Laxton	Daisy
A	127.6	106.3	106.9	105.7
P <sub>5</sub>	139.2	104.5	100.0	101.8
Control	100.0	100.0	100.0	100.0

In the last three trials, the relation of treatment A to the control is very similar, but in no case is the difference statistically significant. In comparing the four experiments, the only definitely significant increase over the control is shown in the case of the autumn sown experiment. In the first experiment the increase shown by the compound P<sub>5</sub> over compound A is no doubt chiefly due to the fact that in the former the rate of application of the dust was at 3 oz. per bushel of seed, whereas in the latter it was only 2 oz. per bushel.

It is of particular interest to compare the four experiments on the basis of the germination in the field expressed as a percentage of the laboratory germination, as in Table X.

Table X. *Germination in the field expressed as a percentage of the laboratory germination*

Treatment	Laxton's Superb	Little Marvel	Thomas Laxton	Daisy
A	63.3	71.0	66.3	88.2
P <sub>5</sub>	69.0	69.8	61.9	84.9
Control	49.6	66.8	61.9	83.4
Date sown	7. xi. 34	13. iii. 35	2. iv. 35	4. vi. 35
Date of count	11. xii. 34	23. iv. 35	7. v. 35	24. vi. 35
No. of weeks after sowing of counts	5	6	5	3
Laboratory germination %	76	99	97	86

For the purpose of comparison, it may be taken that the counts of the later experiments, from the point of view of stage of development

of the crop, are reasonably comparable with the first count made of the Laxton's Superb.

A comparison of the experiments on the above basis shows a marked similarity in the figure for the control in the case of Little Marvel and Thomas Laxton, and it may suggest that for sowings in March and April on heavy soils, the soil germination of a bulk of peas of good quality will approximate to 60–65 per cent of the laboratory germination as determined by the standard sand method. The higher relative soil germination shown in the last experiment—sown on 4 June 1935—is no doubt due to the later time of sowing, when a higher soil temperature and somewhat less moist seed bed would both have some influence. It is unfortunate that in the later experiments bulks of seed comparable with the Laxton's Superb, from the point of view of laboratory germination, were not available, and it is not clear from the results whether the lower soil to laboratory germination ratio, in the case of the first experiment, is due chiefly to the initial lower viability of the seed or to the very early sowing. The fact that the application of the compounds A and P<sub>5</sub>, in this case, increased the ratio of soil to laboratory germination to figures approaching those of the Little Marvel, suggests that the lower ratio of the untreated is due not so much to the quality of the seed as to the handicaps imposed by November sowings on a heavy clay soil.

#### DETAILS OF THE COMPOSITION OF THE DUSTS USED

P<sub>1</sub>. 1 per cent Hg as methyl mercury chloride plus 0.5 per cent Hg as mercuric iodide.

P<sub>2</sub>. 2 per cent Hg as methyl mercury chloride plus 1.0 per cent Hg as mercuric iodide.

P<sub>3</sub>. 3 per cent Hg as methyl mercury chloride plus 1.5 per cent Hg as mercuric iodide.

P<sub>4</sub>. 1 per cent Hg as methyl mercury phosphate.

P<sub>5</sub>. 2 per cent Hg as methyl mercury phosphate.

P<sub>6</sub>. 3 per cent Hg as methyl mercury phosphate.

P<sub>7</sub>. 1.7 Hg as phenol mercury acetate.

In all the experimental dusts the filler was china clay.

The toxicity of this series of mercury compounds, their vesicant action and other properties have been indicated previously<sup>(1)</sup>. If they are used for experimentation, adequate precautions must be observed.



## CONCLUSIONS

The evidence produced by these experiments indicates that for sowings earlier than March, when encountered, the application of a suitable organic mercury compound in the form of a dust is likely to result in an increased stand and yield of marketable pods. For sowings later than this there is evidence that the advantages of the application of such a dressing are doubtful. There is a suggestion, too, that the soil to laboratory germination ratio tends to increase as the date of sowing advances, and may be in the region of 60–65 per cent for sowings in March and April.

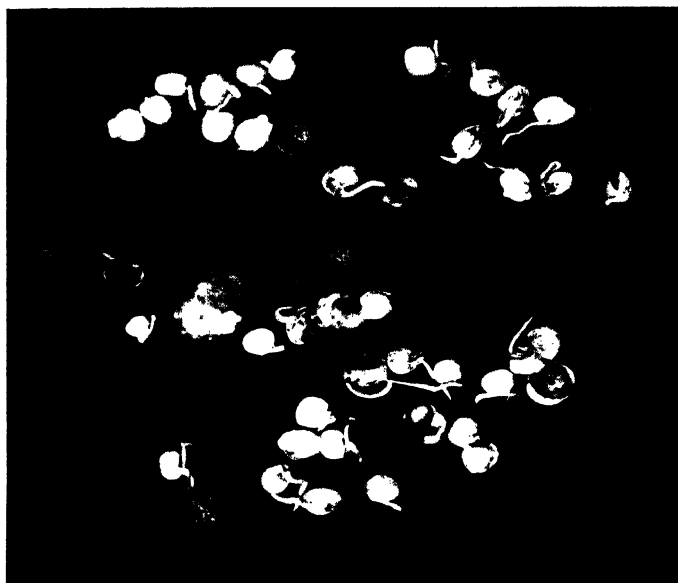
The greenhouse and small-scale field trials produced data to support the view that probably the chief value in the application of an organic mercury compound to peas lies in its protective action.

The writers wish to acknowledge their indebtedness to Mr A. Eastham, of the Official Seed Testing Station, both for his continued interest and advice and for the facilities afforded for doing the laboratory, greenhouse and preliminary small-scale field trials. To Mr W. H. Parker, Director of the National Institute of Agricultural Botany, they are also grateful, particularly for the facilities provided for the carrying out of the field trials. Their thanks are due also to Messrs Charles Sharpe and Co., Ltd., Messrs Cooper Taber and Co., Ltd., and Messrs Hurst and Sons for supplies of seed used in the experiments.

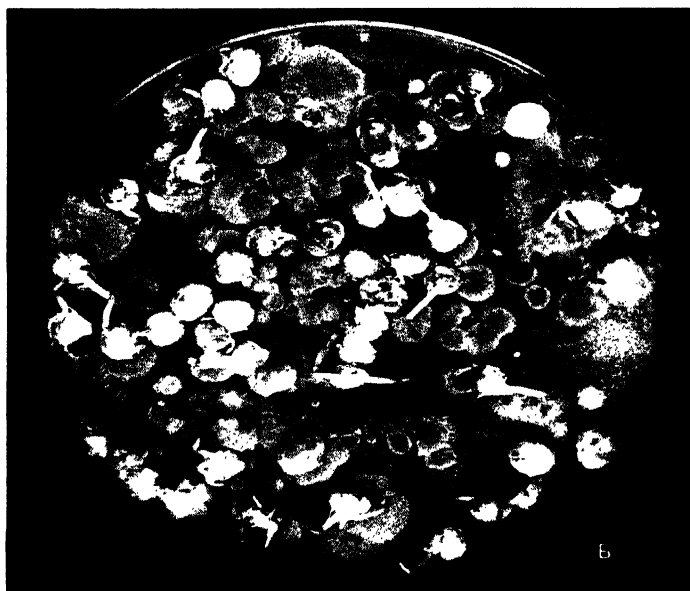
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A. Seed dusted at the rate of 2 oz. per bushel and plated out on sterile potato agar medium. (The slight markings on the medium are not mould growths but traces of the fungicidal dust that have been rubbed off the seed.)



B. Undusted seed plated out on sterile potato agar.



# THE LOSSES OF DRY MATTER AND DIGESTIBLE NUTRIENTS IN LOW-TEMPERATURE SILAGE, WITH AND WITHOUT ADDED MOLASSES OR MINERAL ACIDS

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## INTRODUCTION

IN a recent paper<sup>(1)</sup> the general principles underlying the process of silage making have been considered, together with the main changes in chemical composition. The addition of a readily fermentable carbohydrate (molasses) in order to stimulate lactic acid formation and of mineral acids in amounts calculated to reduce the  $pH$  of the mass to below 4.0 (Virtanen<sup>(2)</sup>), both resulted in the production of silages of excellent quality as judged by their chemical characteristics.

It was concluded that it was necessary to take into account the losses of nutrients before any decision could be made as to the relative efficiencies of these two processes. The ordinary low-temperature process of making silage gave a product which, though never so certain as the two processes mentioned above, nevertheless was very often of good quality if suitable conditions of making were ensured.

The two main points in making good silage are the exclusion of air as much as possible and rapid acidification of the mass, either directly by the addition of acid, or indirectly by the stimulation of a strong lactic acid fermentation, both of which will keep undesirable forms of fermentation in subjection.

During the last five years a number of experiments have been carried out to measure the losses of dry matter and of digestible nutrients involved in the process of ensilage. Unfortunately, owing to a long sequence of dry years it has not proved possible finally to settle the matter of relative losses to our satisfaction until 1935.

## EXPERIMENTAL PROCEDURE

Some of the low-temperature silages have been made in a steel tower silo, but the majority have been made in silos of the type which have been popularized by the work of Virtanen<sup>(2)</sup>. These consist in essence of

an undersilo of any desired diameter, made of wood or concrete, some 5-6 ft. high with a movable oversilo of wood of like diameter. The silo is usually filled to the top of the oversilo, and when the mass has settled, this is removed. It is usual to put a layer of soil on the surface of the herbage to exclude the air and compress the mass. The soil is kept from coming in contact with the silage by a layer of paper or old sacks. The general lines of each of the processes adopted can now be considered.

The material used in our work has normally consisted of grassland herbage, usually from permanent pasture, and containing variable amounts of leguminous plants—for the most part wild white clover, and has never been chaffed.

#### LOW-TEMPERATURE SILAGE

This name has been adopted to distinguish the process from the cold fermentation process in which the crop is kept at a temperature below 80° F., and from the warm fermentation process in which it usually rises to 120° F. and over.

With grassland herbage, which packs tightly, it has proved desirable to let the lower layers heat somewhat before the silo is completely filled, in order to prevent the formation of an undesirable type of silage rich in butyric acid. The aim has been so to control the changes in the silo that the temperature never exceeds 100° F. and lies between 80 and 100° F., a range optimal for the development of most bacteria.

In making low-temperature silage, a layer of variable depth has been filled in during the first day, and this has then been allowed to heat to the desired temperature level, after which the silo was filled up to the top of the oversilo. In the tower silo the succeeding layers are of greater depth as the top is approached. In all cases the mass is covered, and suitable weights applied *immediately* after the last filling.

Care was taken in filling to see that the material was well shaken out to prevent pockets of entangled air, that each layer of about 6 in. was well and evenly trampled into the silo, and that the sealing layer of soil was carefully applied and did keep out air as efficiently as possible. After the mass had settled, the soil cover was readjusted to leave a mushroom-shaped top capable of running off rain water. A simple straw thatch was added in some cases still further to protect the silage.

#### MOLASSED SILAGE

In making silage from young grassland herbage, which is usually relatively deficient in fermentable carbohydrates for lactic acid production, it is useful to add sugar or molasses in solution to overcome

this shortage and stimulate the desired fermentation. The silos were filled exactly as with the low-temperature process, but during filling each shallow layer of 6 in. or so was sprinkled from a watering-can with the sugar solution. Molasses was usually added at the rate of 1-2 lb. per 100 lb. of fresh grass (22.4-44.8 lb. per ton), but the amount was reduced latterly to 15 lb. per ton of fresh grass without any loss in efficiency. The molasses was diluted before use with one to two times its volume of water. It must be emphasized here that it is not essential that the rate of addition be adhered to rigidly, but care should be taken that the layers are not too thick and that the solution comes into contact with the greatest amount of the mass in the silo that is possible. In practice the total addition can be calculated from the capacity of the silo, and the weight of molasses suitably diluted is added to the herbage as equally as is practicable and, in point of fact, owing to the tendency for the solution to leach downwards in the silo, rather more can be added in the uppermost layers.

#### SILAGE MADE WITH ADDED WHEY

A number of silages were made with added whey solutions. Fresh whey, as regards its lactose content, is very dilute, and was used only in one silo. To the other silos whey paste or powder, suitably diluted, was added at a rate sufficient to supply 1 lb. of lactose per 100 lb. of fresh crop. The procedure was identical with that adopted for the low-temperature process with added molasses.

These silages were made in small concrete silos holding 1 ton, but otherwise similar to the larger silos normally used.

#### SILAGE MADE WITH ADDED ACID

The silage made under this heading can be divided into two classes, that made by the A.I.V. process (2) and that made by other acid processes. The underlying principle in both cases is to induce rapidly a sufficiently high degree of acidity in the mass to control undesirable fermentations.

*The A.I.V. process.* This process, introduced by Virtanen (2), depends upon the reduction of the pH of the mass in the silo to below 4.0. The quantity of acid solution to use is calculated beforehand in laboratory tests for experimental purposes, though for practical purposes suitable additions are suggested. The acid used is a mixture of hydrochloric and sulphuric acids (3, 4) of twice normal strength, and the amount added varies with the crop, but is in the neighbourhood of 14 gallons per ton. In this case there is no check to filling, which continues until the silo is full. The crop is filled in layers of not more than 6 in., and each layer

is properly shaken out, trampled, and then sprayed with the *requisite amount of acid*. This is important, and must be adhered to if the whole of the mass is to reach the correct pH level and variable layers are not to be met in the silo. It is usual to weigh each load of grass or to weigh a number of forkfuls and calculate the requisite addition of acid from the known weight in each layer. This, in our opinion, is one of the most serious practical disadvantages to the whole process.

The silos were filled as rapidly as possible, and covered with a layer of paper or sacks as soon as they were filled, and a layer of at least 12 in. of soil then added. When the silage had settled, the oversilo was removed and the soil cover made good in the usual way.

Where, for any reason, filling took more than 1 day, the mass was weighted temporarily overnight by laying planks across the top and putting on as great a weight of readily removable material such as bags of soil and concrete posts as could be obtained.

In the finishing of the silo Virtanen(2,5) has used a special anti-mould preparation consisting chiefly of mustard oil and allied compounds. In comparative trials this surface treatment, where it has been applied in the A.I.V. process, has also been used with other processes. In general, where care is taken in sealing the silo with soil, no particular advantage has been obtained from the use of this preparation.

#### OTHER ACID PROCESSES

The cases in which other types of acid have been used or lower concentrations have been added have been grouped separately. Among these are two in which the principle of the A.I.V. process was adhered to, i.e. the pH was reduced below 4.0. In one, sulphuric acid alone was used at twice normal strength. In the other, phosphorus oxychloride was used in such dilution that the resultant hydrochloric and phosphoric acids were at the same strength as that used in the A.I.V. process.

The remainder of the silages in this class were made with lower additions of acid solutions. In one series, the Defu(6) process was used, in which a dilute solution of hydrochloric and phosphoric acid is added at a rate below that adopted for the A.I.V. process. To this was added a small amount of molasses in some cases. The rate of addition was sufficient to produce a pH of 4.5 in the mass, and the fermentative product of the molasses is relied upon to increase the acidity still further. Finally, Penthesta(7) was used. This is phosphorus pentachloride, a solid which in solution gives phosphoric and hydrochloric acids. It was added in the same way as the Defu solution.

Enough has been said to show how the different types of silage were made.

#### MEASUREMENT OF LOSSES

This is a most difficult process in reality, as we have found from experience.

Analysis of the fresh crop and of the resultant silage gives no information whatever as to the magnitude of the losses, unless this is reinforced by a knowledge of the weight of dry matter put into the silo and that taken out. This should be clearly understood, and if it is realized will do much to clear up a great deal of misunderstanding about the relative values of different processes of silage making. In some cases an attempt has been made to use the crude analytical data to measure the losses. As an example, the assumption has been made that the fibre is unchanged, and on this basis the losses have been worked out from the analysis of crop and of silage. The errors involved in this process are shown in Table I, where the losses have been calculated from the analytical values given later, and are compared with those determined directly.

Table I. *Losses of dry matter calculated from analytical values, on the assumption that the fibre was unchanged, compared with values determined directly (stated as percentages of fresh crop)*

	Low temperature	Molassed silage	Whey silage	A.I.V. fodder	Miscellaneous acid silages
Calculated	10.0	13.4	2.9	11.6	9.4
Determined	18.2	16.1	17.7	17.7	18.0

The calculated value is too low in all cases. It is not to be expected that this method of calculating losses would give any true indication, since Norman (8) has recently shown the shortcomings of the fibre determination. On a *priori* grounds it is obvious that fermentation must result in some change in the fibrous constituents of the mass.

A knowledge of the digestibility of the material does afford some clue to losses if large differences appear between the values for the fresh crop and for the silage, but for comparing different processes this is of little value. It is only where the losses are exceedingly high that very marked differences in digestibility are found. There is usually some diminution in digestibility, as will be seen later, owing to the fact that the material lost in respiration and fermentation is always the most readily digestible, so the residue must be of lower digestibility.

Similarly a comparison of the digestible nutrients or starch equivalent in the fresh crop with those in the silage will not give a numerical measure



of the losses. In comparing a really bad silage with a good silage and the fresh crop, it will be obvious that the first will be of lower feeding value, since the processes in the silo have not been properly controlled, and the loss of highly digestible material, and hence nutritive value, is greater, but such a comparison will give no information as to the magnitude of the losses.

The losses in the silo may and do affect *all the constituents*, the only one which shows any increase being the ether extract which includes the organic acids resultant on the chemical changes. The ether extract forms but a small part of the dry matter, and it is possible for all the other constituents to suffer a loss, and the individual losses may not differ greatly in some cases, so the analysis of the silage is almost identical with that of the fresh crop. This is an exaggerated case but, nevertheless, the principle holds good.

The size of silo used is another important factor. In general, laboratory experiments give misleading results which are not applicable to practice. In small silos, such as the small steel cylinders used on the Continent<sup>(9)</sup> with capacities of a few cwt. and no drainage, it is possible to control respiration so carefully that losses are extremely low. On a larger scale this is not possible. If a long crop is being used in a small container, the opposite may be the case, it becomes impossible to compress properly as the material gets entangled and clings to the side. As it settles, air is drawn down and the mass rises to a very high temperature with coincident high losses, and moulds may enter and cause still further losses.

The experiments on silage with added whey were carried out in silos which held 1 ton of fresh grass, and were made of concrete with a removable steel oversilo. The smooth inside walls allowed the grass to settle, and the crop itself was not too long, and good silage resulted.

We have also used larger wooden silos at Jealott's Hill, each with a capacity of 2 tons of fresh crop. Difficulty was always being experienced with this type. The losses were of a higher order than were expected, and the material never seemed to settle properly. The diameter (6 ft.) was so small that the side-cling of the material on the walls affected the greater part of the silage in the container. The wooden sides of the undersilo, which was sunk to ground-level, were also pervious, and water seepage was found to be responsible for some of the anomalous results. To check the question, five silos were filled from the same field at the same time and in the same way. The silos were filled on 16 June and opened on 14 November of the same year, and the losses of dry matter were determined. The first silo was found to have a high proportion of

slimy dark material at the sides, which it was impossible to divide from the good silage. The second silo appeared fairly good, but there was a distinct odour of butyric acid, the silage was fairly wet, and there was considerable loss at the sides.

The third silo was better, but patchy, with a little mould on top, but the sides were still of poor quality.

The fourth silage was of good colour and pleasant smell. The silage appeared to be slightly drier. The fifth silo produced the best silage, with very little mould.

The actual losses of dry matter were as follows:

Table II. *Losses of dry matter in five small wooden silos filled with the same grass and in the same way (percentage of original grass)*

Silo	%
1	30.5
2	31.1
3	22.2
4	22.5
5	13.7

The results from silo 5 agreed with figures obtained for a large silo filled with grass from the same field at the same time. It is obvious from the table that seepage had contributed to the wide variation. This has never happened with concrete pits. As a result, all our experimental work has been carried out in concrete-lined pits or, where wood has been used, the silos have never been sunk their full depth, the portion above ground being banked up with soil where necessary. The value for silo 5 shows that there are conditions under which the small wooden silo can be used when sunk wholly into the ground, but the conditions have to be carefully standardized before they can be used with confidence for comparative work.

It is our considered opinion that where nutritive losses are to be measured, the silo should hold at least 8–10 tons of silage. This gives conditions in the silo which approximate to practice, and this is what is required.

The next point at issue is whether or not the whole of the contents should be weighed. A great deal of work has been done by the "bag method", in which a bag containing a weighed amount of the crop is placed in the silo. Though, doubtless, very satisfactory results may be obtained by this technique, it is often liable to difficulty. In some cases air is entangled in the bag, local high temperatures are met with, and all investigators have been faced with the difficulty that as a result it is sometimes impossible to recover every bag. Then again, there is the

leaching in the silo to be taken into account; the upper bags will be found to have suffered losses in certain constituents, such as the ash, whilst the lower bags will show a gain in this constituent. The average value for the bags is not necessarily a true measure of the mean losses.

But the chief point about this method of measuring losses is that it takes no account of the losses which do occur at the top and at the sides of the silo due to slight inferiority in quality, since the exclusion of air and control of fermentation cannot be so complete there. The bag method was said by one of the earliest workers in this field (10) to measure the "unavoidable losses", by which he referred to the respiration and fermentation losses as opposed to losses due to poorer quality silage at side and surface which must, however, be taken into account.

In two small silos at Jealott's Hill bags were used, and losses determined by the "bag method" and by weighing the contents of the silos.

Table III. *Losses of dry matter obtained by the bag method and by weighing all the contents of the silo (percentage of fresh crop)*

Silo	Based on silo contents	Based on bag weight	Difference in favour of bag
1	21.1	(i) 15.2 (ii) 8.5	+5.9 +12.6
2	21.2	(i) 16.1 (ii) 11.6	+5.1 +9.6

The figures call for no comment, the lower bags show lower losses in both cases, and all bags show lower losses than were registered in the silos.

To get the most accurate figures, the whole contents of the silo should be weighed. In ordinary practice, a silo is emptied over a relatively long period, and during the emptying there is a variable drying out of the surface layers which makes sampling a somewhat difficult procedure. To avoid this, we have usually emptied our silos at Jealott's Hill in one day, though in earlier experiments the normal method of emptying was adopted and special care taken to mix each load removed before sampling for dry-matter determination.

The material removed from a silo after being weighed and sampled was packed into another empty container, and it was found that no great harm resulted, and the silage could be fed out later as desired, no undue moulding taking place where it was firmly trampled down.

The whole value of experiments in measuring nutritive or dry-matter losses depends upon the accuracy with which the dry-matter content of the fresh crop and the silage is determined. An adequate number of samples must be taken, and due precautions adopted to make these

values as far as possible representative. We have come across cases in which the estimate of losses was based on a single sample taken on one day from a mass of silage, fed out over weeks. A crop used for silage will usually contain between 70 and 80 per cent of moisture. An error of 1 or 2 units is not large when compared with the water in the crop, but is appreciable when referred to the dry matter, and this is, of course, the basis of comparison. In an experiment published recently<sup>(4)</sup>, and which will be referred to later, the moisture content of the crop varied from 87.37 to 89.04 per cent. This is very high. More than 11 tons were filled into the silo, from which three samples were taken for moisture determination, giving 10.94, 12.63 and 12.77 per cent of dry matter, with an average of 12.10 per cent. The average is arithmetical, and had the error in this value been 0.5 it would mean an underestimate of the dry matter put into the silo of some 4–5 per cent. Anyone who has worked with silage will realise the difficulty of sampling material which contains but 12 per cent of dry matter, especially when three samples are to be drawn from a mass of over 11 tons.

Furthermore, the figure for dry matter should be a weighted mean where a number of samples are taken.

#### SAMPLING OF FRESH GRASS. TECHNIQUE ADOPTED IN EXPERIMENTS

We have made a practice of taking a sample from each load. Each load of fresh grass is weighed on the farm weighbridge prior to being forked into the silo. The loads are weighed to the nearest 7 lb. A sampler is put at a suitable position, and as the fresh crop is being transferred to the silo he takes small subsamples from each forkful as it is thrown into the silo. These can be taken with the finger and thumb very readily. At the end of the load a sample of 2–3 lb. will have been collected.

This is immediately taken to the laboratory, cut up in a small chaff-cutter, and subsampled in duplicate for dry matter determination, 200 g. of material being used for each determination. The dry matter is determined in an oven operating at 96° C. with a hot air circulation system which dries the sample in 4–5 hours. It is weighed off at constant weight, ground and bottled. Each load is sampled separately, and the dry matter bottled separately.

Bartlett & Greenhill<sup>(11)</sup> have shown that in sampling heaps of grass there was little advantage to be gained from duplicate sampling or subsampling in reducing the experimental error for dry matter determination, since 65 per cent of the error is due to the difference between plots (or loads in this case).

The subsampling in duplicate is useful as a check against any gross error in manipulation or calculation.

The process is best illustrated by a concrete example, and for this purpose a molassed silage will be taken.

Table IV. *Weight of fresh grass filled into the silo*

Load No.	Date	Fresh weight			Dry matter	
		cwt.	qr.	lb.	%	lb.
1	May 23	20	3	0	22.98	533.6
2	"	23	1	7	23.00	600.5
3	"	23	0	0	24.45	629.8
4	"	25	0	14	23.42	659.0
5	"	23	1	14	23.20	607.4
6	May 24	20	3	21	25.95	608.0
7	"	23	0	14	27.70	717.4
8	May 25	33	3	14	19.55	741.7
9	"	32	2	0	19.22	699.6
10	"	18	1	14	19.18	394.7
Total		244	1	14	22.62*	6191.7
Molasses added		1	2	27	74.00	144.3

\* Weighted mean.

#### EMPTYING THE SILO

The silo was opened on 9 September and contained a good sample of silage. It was emptied in one day, the silage being forked off in layers after the careful removal of the soil and the sacks. There was very little waste on top, and this was confined to a blackened layer in contact with the sacks. This was scraped off with a spade, and was of negligible weight. As each load was removed, a sampler took small samples from each forkful and placed them in a bag as with the fresh grass. As each load was emptied, the sample was taken to the laboratories, subsampled in duplicate for dry-matter determination, and the remainder of the sample kept for making a composite sample for pH determination. The sample was chaffed for this purpose.

The figures obtained with the silage in question are given in Table V.

The last load consisted of the material from the top of the silo. It was very slightly mouldy, but the damaged material was too little to separate. It was feared that it might differ in moisture content, and was sampled separately. By comparison with load 1 it will be seen that this separation was necessary. The whole of the silage in load 12 was eaten by stock. The sheep feed was made up of layers cut out at different depths for use in the metabolism trial with sheep, and was weighed and sampled separately.

Table V. *Weights of fresh silage and dry matter removed from the silo*

Load No.	Fresh weight			Dry matter	
	cwt.	qr.	lb.	%	lb.
1	16	2	0	19.18	353.4
2	16	1	7	17.85	325.3
3	15	3	7	19.85	351.6
4	16	2	21	21.45	399.7
5	17	1	14	22.85	443.5
6	15	3	7	22.93	406.1
7	20	2	0	21.68	497.8
8	16	0	14	20.92	378.2
9	15	1	21	21.77	376.3
10	20	0	14	21.80	491.5
11	23	3	21	22.28	578.6
12*	5	0	7	18.20	103.2
Sheep fed	2	1	21	22.03	60.2
Total	202	0	14	21.05†	4765.4

\* Load from top slightly mouldy but all eaten by stock.

† Weighted mean.

#### ACCURACY OF SAMPLING

To check the method of sampling adopted, it was necessary to know the errors involved when silage is being removed from a silo.

An experiment was carried out in 1935 to determine the magnitude of this sampling error.

When the contents of each of two circular wooden pit silos were being emptied, the normal practice of taking one representative sample from each load was replaced for several loads by sampling in triplicate with three different samplers. To compare the variations in samples so obtained with the subsampling error corresponding to duplicate determination of dry matter in the laboratory, the samples from the remaining loads were analysed in duplicate for dry matter: a single dry-matter determination only was made for each of the triplicate field samples.

We are indebted to Mr M. S. Bartlett for the statistical analysis and comments on the data obtained from the two tests, one of which was conducted on an A.I.V. fodder, the other on the molassed silage dealt with in Table V.

The first test was carried out with three samplers, two outside the silo, one on each side of the cart, the other in the silo (C).

The second test was carried out with three samplers all outside the silo, standing at different points alongside the cart, B and C being on one side, A on the other.

The results of the tests are given in Table VI.

Table VI. *Dry-matter content of samples taken by separate samplers, and duplicate samples on remaining loads (stated as percentages)*

Silo	Load No.	Separate sampling			Load No.	Duplicate determinations	
		A	B	C			
A.I.V. fodder	5	19.80	20.50	20.80	1	20.55	21.65
	7	21.25	22.10	21.90	2	21.00	21.20
	8	22.45	22.70	23.15	3	20.40	20.35
	9	22.45	22.55	23.40	4	20.40	20.00
	10	22.85	22.80	22.80	6	19.95	21.15
	Mean	21.76	22.13	22.41	11	23.85	22.95
					12	22.75	22.65
Molassed silage	5	23.00	22.50	23.05	1	19.90	18.95
	6	22.85	22.85	23.10	2	18.05	17.65
	7	21.60	21.70	21.75	3	19.75	19.95
	8	20.85	21.10	20.80	4	21.05	21.85
	9	21.50	21.90	21.90	10	21.65	21.95
	Mean	21.96	22.01	22.12	11	21.80	22.75

The results of an analysis of variance are given below.

Table VII. *Analysis of variance*

	A.I.V.			Molasses		
	D.F.	Variance	S.E.	D.F.	Variance	S.E.
Duplicates:						
Between duplicates	7	0.2623	0.5122 (2.40 %)	6	0.2279	0.4773 (2.34 %)
Sampling:						
Among samplers	2	0.5315	—	2	0.0335	—
Interaction	8	0.0821	0.2865 (1.30 %)	8	0.0412	0.2030 (0.92 %)

No differences are apparent between samplers for the molassed silage, but the variation between them, when compared with the interaction term between samplers and loads, is significant ( $P < 0.05$ ) for A.I.V. fodder, the value of  $z$  being 0.9340. The magnitude of any bias between them was, however, small, being for a single sample of the order of 0.2998 (1.36 per cent of the general mean).

It will be noticed that the variation between duplicates is in each case larger than the interaction term between samplers and loads, significantly so for molassed silage ( $z = 0.8552$ ,  $P < 0.05$ ).

This result would hardly be expected, since any such subsampling error in the laboratory should be implicit in the variation for the separate field sampling errors. Moreover, the errors between duplicates obtained here compare favourably with the usual errors experienced previously. The apparent reduction in these errors might be explained by the extra

care given to the work. The standard error corresponding to the error between duplicates was 2.4 per cent of the general mean.

The smallness of the interaction terms indicate that there is no evidence at all for random field sampling errors, as distinct from the question of bias.

The above figures for dry matter, as with those in Table V, take no account of the volatile substances lost during the drying of the sample, but a correction has to be made for this in the analytical determinations.

It has been stated that after subsampling the load samples for dry-matter determination, the remainder was retained for pH determination. Three composite samples were made from all the loads. Each sample was a true weighted sample based on the weight of the fresh crop in each load. The dry matter of the composite samples was determined, the pH estimated with a quinhydrone electrode. The total acidity, volatile bases, amino acids and volatile acids were determined by Woodman's modification (12) of Foreman's method (13). In addition the volatile bases and volatile acids were determined on composite samples made up from the dry matter of the loads of silage in the same way as the composite samples of fresh silage. The difference between the figures gives the percentage of volatile acids and volatile bases lost during the drying of the sample, figures necessary to the correction of the dry-matter figures. The figures are summarized in Table VIII.

Table VIII. *Dry-matter content, pH value, total acidity, volatile base, amino acid and volatile acid content of molassed silage (percentage of fresh silage)*

Layer	pH	Dry matter %	Total acidity as c.c. N/10 acid	Volatile* bases	Amino* acids	Volatile acids as acetic acid	Percentage losses on drying	
							Volatile bases	Volatile acids
Top	4.17	20.7	521.4	0.37	1.52	0.78	—	—
Middle	4.10	22.5	500.5	0.30	1.21	0.69	—	—
Lower	4.07	21.5	482.0	0.25	1.08	0.62	—	—
Average	4.11	21.6	501.3	0.31	1.27	0.70	38.7	85.6

\* Stated as crude protein N  $\times$  6.25.

#### COMPOSITION AND DIGESTIBILITY OF FRESH GRASS AND MOLASSED SILAGE

The dry matter obtained from the samples from each load was ground and bottled. These separate load samples were then used to make up a composite sample of the dry matter in the fresh crop put into the silo and also of the silage removed. The composite samples are each weighted for the amount of dry matter actually found in each of the loads.



These composite samples were analysed in the usual way by the methods in general use. The digestibility of the fresh grass was determined with a pair of sheep fed on grass cut from the area to be made into silage for a week before the date of cutting, and for a week after from strips left in the field at random for this purpose.

The digestibility of the silage was also determined on the special sheep feed which has already been mentioned, and which was made up from the layers picked from all parts of the silo and made into a block from which the daily feeds were taken, steps being taken to ensure that all parts of the silo contributed to the diet.

The figures for composition, digestibility and digestible nutrients of the silage are given in Table IX.

Table IX. *Composition, digestibility and digestible nutrients in the fresh grass and the silage made from it (stated as percentages of the dry matter)*

	Silage						
	Fresh grass			Composition			
	Com- position	Digesti- bility	Digestible nutrients	As deter- mined	Corrected for volatiles	Digesti- bility	Digestible nutrients
Ether extract	3.63	59.9	2.17	5.08	7.66	79.8	4.05
Fibre	20.56	85.2	17.52	24.84	24.02	87.6	21.04
Crude protein	16.04	78.9	12.66	16.56	16.57	73.7	12.20
Ash	8.62	—	—	10.24	9.90	—	—
N-free extractives	51.15	85.4	43.68	43.28	41.85	81.3	34.02
Organic matter	91.38	83.1	75.94	89.76	90.10	81.3	73.25
“True” protein	13.21	76.0	10.04	9.00	8.70	46.6	4.05
Calcium (CaO)	0.70	—	—	0.86	0.83	—	—
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.85	—	—	0.75	0.73	—	—
Ratio “True” protein Crude protein	0.82	—	—	0.54	0.53	—	—
Starch equivalent	—	—	68.5	—	—	—	58.6
Starch equivalent (corrected)	—	—	—	—	—	—	63.8
“True” protein (corrected)	—	—	—	—	—	—	9.64
Protein equivalent	—	—	11.35	—	—	—	8.13
Protein equivalent (corrected)	—	—	—	—	—	—	10.92
Dry matter in fresh crop	22.62	79.2	—	21.05	24.46	77.4	—

The values for the fresh crop are straightforward, and call for no comment. The starch equivalent has been calculated by the method advocated by Kellner(14), using the digestible “true” protein for calculating the energy due to nitrogenous substances and adopting the usual correction factor for fresh grass with such a fibre content. The protein equivalent is the average of the digestible crude protein and digestible “true” protein values, as usually calculated(15). Some

explanation has to be made for the analytical values for the silage. These have to be corrected for volatile constituents lost in the drying of the sample. This is done on the basis of the average values in Table VIII. The volatile bases lost, stated as crude protein, are added to the crude protein fraction; the volatile acids lost, calculated as acetic acid, being added to the ether extract, in which fraction they would normally appear. This raises the sum of the constituents to above 100, so they are all calculated down to the correct level. No change is necessary in the "true" protein values other than the reduction to bring the sum of all constituents down to 100. The organic matter has to be corrected for the volatile bases and volatile acids lost on drying before being reduced to the correct level.

The dry-matter content is corrected for volatiles lost.

The digestible nutrients in the silage are calculated in the usual way, with the exception of the digestible ether extract. This is calculated from the value determined on analysis and not the value corrected for volatile acids lost on drying, since these latter are of low nutritive value and cannot rank with the true fat.

A noticeable feature of the silage process is the breakdown in "true" protein as measured by the ratio of this constituent to crude protein.

Modern work has shown that the breakdown products of the "true" protein are of high nutritive value if the process has not proceeded too far, and this has been demonstrated by the authors<sup>(16)</sup>. It is, therefore, more accurate to make allowance for this fact, and the corrected digestible "true" protein value is designed to meet the case. It is calculated from the digestible crude protein by applying to it the ratio which existed in the fresh grass between digestible crude and digestible true protein.

The starch equivalent and protein equivalent values are then recalculated, using the corrected digestible "true" protein value in place of the figure for digestible "true" protein to give the corrected values for the two first-named terms.

#### LOSSES OF DRY MATTER AND DIGESTIBLE NUTRIENTS

Having obtained values for the crude and digestible nutrients in the silage, the losses of these various constituents can now be calculated. The dry-matter balance should first be adjusted for losses in drying, as shown in Table X.

By applying the analytical values in Table IX to the weights of dry matter in and out of the silo, the losses of crude and digestible nutrients can be calculated as under.

Table X. *Weights of dry matter filled in and removed from silo*

	lb.
Weight of dry matter in, as grass ... ..	6191.7
Weight of dry matter in, as molasses ... ..	144.3
Total in	6336.0
Weight of dry matter out, uncorrected ... ..	4765.4
Add correction ... ..	162.5
Total out	4927.9
Difference (loss of dry matter)	1408.1
Loss of volatiles per 100 lb. of dry matter out of silo:*	
Volatile bases as crude protein ... ..	0.570
Volatile acids as acetic acid ... ..	2.841
Total	3.411

\* Calculated from Table VIII, applying percentage losses on drying.

Table XI. *Balance sheet showing losses of crude and digestible constituents in molassed silage (stated in lb.)*

	Grass in		Silage out		Difference			
	Crude lb.	Digestible lb.	Crude lb.	Digestible lb.	Crude		Digestible	
					lb.	%	lb.	%
Dry matter	6191.7	4903.8	4927.9	3814.2	-1408.1	-22.2	-1205.0	-24.0
	144.3*	115.4*						
Ether extract	224.8	134.7	377.5	301.2	+152.7	+67.9	+166.5	+123.6
Fibre	1273.0	1084.6	1183.7	1036.9	-89.3	-7.0	-47.7	-4.4
Crude protein	993.1	783.6	816.6	601.8	-182.4	-18.3	-184.8	-23.5
	5.9*	3.0*						
Ash	533.7	—	487.9	—	-58.5	-10.7	—	—
	12.7*							
N-free extractives	3167.1	2704.7	2062.2	1676.6	-1230.7	-37.4	-1128.7	-40.2
	125.8*	100.6*						
Organic matter	5658.0	4701.8	4440.0	3609.7	-1349.6	-23.3	-1197.4	-24.9
	131.6*	105.3*						
"True" protein	817.9	621.6	428.7	199.8	-389.2	-47.6	-421.8	-67.9
Calcium (CaO)	43.3	—	40.9	—	-3.0	-6.8	—	—
	0.6*							
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	52.6	—	36.0	—	-16.7	-31.7	—	—
	0.1*							
Non-protein nitrogenous substances	175.2	162.0	387.9	402.0	+206.8	+114.2	+240.0	+148.1
	5.9*							
Starch equivalent	—	4241.3	—	2887.7	—	—	-1453.1	-33.5
		99.5*						
Starch equivalent (corrected)	—	—	—	3144.0	—	—	-1196.8	-27.6
"True" protein (corrected)	—	—	—	475.0	—	—	-146.6	-23.6
Protein equivalent	—	702.8	—	400.6	—	—	-305.1	-43.2
		2.9*						
Protein equivalent (corrected)	—	—	—	538.1	—	—	-167.6	-23.7

\* Supplied by the molasses.

This is a typical example, and shows the method adopted in calculating the results of all our trials. There is one correction which might have been made, and that for the alcohol present as a result of the changes in the silo. This constituent has, however, not been estimated, and no correction is possible. Any alteration would be to reduce the losses of dry matter slightly. Since silage is assumed to contain about 0.25 per cent of alcohol<sup>(17)</sup> on the fresh weight, this can at the most reduce the losses by 1-1.5 per cent. In considering the figures to be presented for different experiments, it should be realized that the procedure illustrated above has been followed out in all cases. The complete data are available for inspection by anyone interested<sup>(18)</sup>.

For comparative purposes we have collected together all the experimental data available, and these include seven trials with low-temperature silage, seven with molassed silage, nine with the A.I.V. process, four with added whey, and six miscellaneous acid treatments. The main details are given below in an abbreviated form. For simplicity the low-temperature silage without addition will be called ordinary silage.

#### *Ordinary (low-temperature) silage*

No. 1. Made June 1932 in a small concrete pit from grass at a medium stage of growth. 21 cwt. of grass used.

No. 2. Made June 1932 in a low steel tower from grass at the short hay stage. 73 cwt. of grass used.

No. 3. Made November 1934 in a large concrete pit from young grass. 303 cwt. of grass used.

No. 4. Made May 1934 in a large concrete pit from young grass. 241 cwt. of grass used.

No. 5. Made May 1933 in a small concrete pit from fairly leafy young grass. 18 cwt. of grass used.

No. 6. Made October 1932 in a steel tower from young grass. 206 cwt. of grass used.

No. 7. Made May 1933 in a small concrete pit from fairly leafy young grass. 17 cwt. of grass used.

#### *Silage made with added molasses*

No. 1. Made May 1935 in a large concrete pit from grass at a medium stage of growth. 254 cwt. of grass used.

No. 2. Made May 1935 in a large wooden pit silo from grass at a fairly advanced stage of maturity. 201 cwt. of grass used.

## 84      *Losses of Dry Matter and Nutrients in Silage*

No. 3. Made June 1933 in a small concrete pit from fairly mature grass. 15 cwt. of grass used.

No. 4. Made June 1932 in a small concrete pit from grass at a medium stage of growth. 18 cwt. of grass used.

No. 5. Made May 1935 in a large concrete pit from fairly young grass. 248 cwt. of grass used.

No. 6. Made May 1935 in a large wooden pit silo from young grass. 246 cwt. of grass used.

No. 7. Made May 1934 in a large concrete pit from young grass. 238 cwt. of grass used.

### *A.I.V. fodder*

No. 1. Made May 1934 in a large wooden silo from grass at an advanced stage of maturity. 205 cwt. of grass used.

No. 2. Made May 1934 in a large concrete silo from grass at an advanced stage of maturity. 266 cwt. of grass used.

No. 3. Made July 1932 in a large concrete silo from fairly mature cocksfoot (*Dactylis glomerata*). 339 cwt. of grass used.

No. 4. Made May 1934 in a large concrete silo from grass of fairly good quality grass. 248 cwt. of grass used.

No. 5. Made May 1934 in a large wooden silo from grass of fairly good quality. 223 cwt. of grass used.

No. 6. Made October 1932 in a large metal tower from fairly young grass. 394 cwt. of grass used.

No. 7. Made May 1933 in a small concrete silo from fairly leafy grass. 24 cwt. of grass used.

No. 8. Made May 1934 in a large concrete silo from leafy young grass. 284 cwt. of grass used.

No. 9. Made November 1934 in a large concrete silo from leafy young grass. 241 cwt. of grass used.

### *Silage made with added whey*

No. 1. Made June 1933 in a small concrete silo from grass at a fairly advanced stage of growth. Fresh whey added with lactic acid culture. 18 cwt. of grass used.

No. 2. Made June 1933 in a small concrete pit from grass at a fairly advanced stage of growth. Dried whey added in solution. No culture. 16 cwt. of grass used.

No. 3. Made June 1933 in a small concrete pit from grass at a fairly advanced stage of growth. Dried whey added as a solid. Lactic acid culture used. 16 cwt. of grass used.

No. 4. Made June 1933 in a small concrete pit from grass at a fairly advanced stage of growth. Dried whey added in solution with a lactic acid culture. 17 cwt. of grass used.

*Miscellaneous acid treatments*

No. 1. Made July 1932 in a large concrete pit from grass at a fairly advanced stage of growth. Penthesta ( $\text{PCl}_5$ ) added in solution with sugar according to manufacturers' directions. 243 cwt. of grass used.

No. 2. Made May 1933 in a small concrete pit from fairly young grass. The A.I.V. acid mixture was added at a reduced rate sufficient to give a pH of 4.5. 21 cwt. of grass used.

No. 3. Made May 1933 in a small concrete pit from young grass. Defu solution ( $\text{HCl} + \text{H}_3\text{PO}_4$ ) added, with a little molasses, at a rate sufficient to give a pH of 4.5 in the mass. 20 cwt. of grass used.

No. 4. Made May 1933 in a small concrete pit from young grass. As for No. 3, replacing the phosphoric acid by mono-ammonium phosphate. 19 cwt. of grass used.

No. 5. Made October 1932 in a large concrete pit from leafy grass. Sulphuric acid alone was used at 2N strength to give a pH of under 4.0. 272 cwt. of grass used.

No. 6. Made October 1932 in a large concrete pit from leafy grass. A solution of phosphorus oxychloride (giving a strength of 2N) was added to give a pH of under 4.0. 253 cwt. of grass used.

In this group Nos. 5 and 6 might well have been included with A.I.V. fodder, as they essentially belong to that category, though the acids used differ from the standard solution.

COMPOSITION AND DIGESTIBILITY

It is not proposed to deal with the analyses of the individual silages, but the average values for each group have been calculated and are given in Table XII. The individual values are available for inspection<sup>(18)</sup>, as has already been stated. The variation will be seen, in so far as the crude protein values are concerned, in Table XVII, which tabulates individual losses.

With the exception of the silage made with added whey, where the grass was at a fairly advanced stage of maturity throughout, the crude protein was fairly high. It is lowest in the molassed silage group, but as will be seen later there were four of the seven silages in this group with very low crude protein contents, and these have depressed the average value considerably. The other three averaged well over 16 per cent of crude protein in the dry matter.

Table XII. *Average composition of fresh grass and of silage made therefrom by different processes (stated as percentages of the dry matter)*

Type	Ordinary		Molassed		A.I.V.		Whey		Miscellaneous acid treatments	
	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage
Ether extract	2.70	7.69	2.83	6.46	2.99	6.06	2.61	5.58	2.65	6.80
Crude fibre	23.24	25.56	22.37	25.37	22.43	25.02	24.00	24.69	22.66	24.78
Crude protein	15.22	17.63	13.15	14.05	15.12	16.96	10.70	13.27	15.62	17.93
Ash	9.32	11.94	8.40	10.06	8.91	10.72	7.81	9.41	9.01	10.23
N-free extractives	49.52	37.18	53.25	44.06	50.55	41.24	54.88	47.05	50.06	40.26
Organic matter	90.68	88.06	91.60	89.94	91.09	89.28	92.19	90.59	90.99	89.77
"True" protein	13.38	7.88	11.19	7.50	13.07	10.40	9.17	7.17	13.92	10.64
Calcium (CaO)	0.73	0.96	0.68	0.94	0.67	0.74	0.80	1.09	0.76	0.91
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.77	0.74	0.76	0.70	0.79	0.72	0.67	0.80	0.77	0.85
Ratio "True" protein Crude protein	0.88	0.45	0.85	0.53	0.86	0.61	0.86	0.54	0.89	0.59
Dry matter in original material	22.2	22.3	24.5	24.6	22.5	23.9	26.0	26.0	22.2	22.2

The ether extract shows an increase in the silage in all cases, though this is least in the A.I.V. fodder. The fibre shows an increase throughout, but this is mainly due to decreases in nitrogen-free extractives, a feature of all types of silage.

The crude protein shows an increase in all cases, as does the ash. These, however, are all changes due to the loss of some of the other constituents. The biggest differences are seen in the "true" protein content. All the processes have resulted in a breakdown of a part of this constituent. This is best seen from the ratio of "true" to crude protein. The beneficial effect of the addition of mineral acid, as in the A.I.V. process and the other acid treatments, is clear to see, but even in these cases there is a considerable breakdown.

The effect of acid treatment on calcium content is clearly seen, but it should be noted that the product is as rich in this constituent as the fresh grass. Unfortunately what calcium has been lost will be the most readily assimilable, and hence most readily leached fraction.

The digestibility coefficients can be studied next, and give some valuable information. The best all-round criterion is the digestibility of the organic matter. This has not varied much with the different types of fresh grass used. It is obvious from a comparison of the values for the fresh grass with those of the silage that the process has given a product of equally high digestibility, the differences being within the range of experimental error in metabolism trials.

The ether extract shows an increased digestibility in all cases, but this is only to be expected in view of the greatly increased content of soluble organic acids in this fraction in the silage.

Table XIII. *Digestibility coefficients of fresh grass and different types of silage made therefrom (stated as percentages)*

Type	Ordinary		Molassed		A.I.V.		Whey		Miscellaneous acid treatments	
	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage
Dry matter	74.5	70.2	75.3	75.2	74.3	72.2	73.3	76.7	73.6	70.6
Organic matter	77.0	74.7	78.3	79.2	77.5	77.1	75.2	78.9	76.0	74.7
Ether extract	54.2	66.7	49.8	70.4	53.5	64.4	35.1	69.8	55.7	62.8
Fibre	78.7	82.2	80.7	84.0	79.4	82.2	75.1	82.6	76.8	80.6
N-free extractives	79.1	71.9	81.2	80.1	79.8	76.7	78.9	79.8	77.7	72.7
Crude protein	72.3	70.0	68.5	69.0	71.5	73.3	66.3	70.2	74.2	73.7
"True" protein	70.0	44.5	65.1	43.3	68.4	60.0	63.0	53.2	71.7	58.2

The ensilage process has increased the digestibility of the fibre, and to some extent that of the crude protein. The nitrogen-free extractives do not show much change, except in the case of the ordinary silage and miscellaneous acid treatments, but these differences are not very marked. The greatest differences are to be seen in the depression of the coefficients for the "true" protein and the advantage of the acid treatments, and the A.I.V. process in particular, is striking in this regard.

#### DECOMPOSITION PRODUCTS OF SILAGE CHANGES

The *pH* of the silage is the best index of its value. The average values for the different groups of silage are tabulated below, together with the figures for total acidity, volatile base, amino acid and volatile acid contents. The residual acidity is also summarized in terms of lactic acid. This residual acidity is the difference between the total acidity and the sum of the amino acid and volatile acid acidities.

Table XIV. *pH, volatile acidity, volatile base, amino acid and volatile acid contents of different types of silage (stated on the basis of the fresh silage)*

Type	...	Ordinary	Molassed	A.I.V.	Whey	Miscellaneous acid treatments
<i>pH</i>		4.65	4.09	3.76	3.86	3.96
Total acidity c.c. N/10		395	462	360	432	375
Volatile bases as crude protein %		0.59	0.27	0.24	0.21	0.33
Amino acids as crude protein %		1.05	1.04	0.72	0.99	0.93
Volatile acids as acetic acid %		0.79	0.64	0.47	0.52	0.51
Residual acidity as lactic acid %		1.30	2.12	1.79	2.09	1.65

The *pH* average values show that the ordinary silage has been the least acid. The A.I.V. fodder has conformed to the desired conditions and averages under *pH* 4.0. The average value for molassed silage is not much over this figure, and the silages made with added whey were also very satisfactory. The value for *pH* in the miscellaneous acid treatments



is lowered by two silages, Nos. 5 and 6, which might well have been included in the A.I.V. treatment, since they were made on the principles laid down by Virtanen(2).

The values for total acidity agree in the main with the *pH* values, except where the more readily dissociated mineral acids were added, these giving, despite their low *pH* value, low values for titratable acidity.

The residual acidity is interesting. In the silage with added molasses and whey the figures are considerably higher than in the ordinary silage, which accounts for the better control of the changes in the two former types, and hence the better quality. The residual acidity in these three types has been found to consist almost entirely of lactic acid(1), but in the silages made with added mineral acids this is not the case. Here again, despite the lower *pH* values, the residual acidity is low in the A.I.V. and miscellaneous acid treatments.

The volatile acids were relatively low throughout, but the A.I.V. process showed the lowest values for this fraction; evidence of its greater control of the breakdown of organic matter in the silo. The volatile acids, as determined by Foreman's methods, are calculated as acetic acid, but also include any butyric acid which may be present. The chemical study of a large number of silage samples already mentioned(1) has shown that the lower value of A.I.V. fodder for volatile acids is due in the main to a lower actual content of acetic acid, and not necessarily to the presence of butyric acid in other types, although ordinary silage is liable to contain larger amounts than any other type. The fractionation of the volatile acids was not carried out in all cases, but in the later samples Wiegner's(19) method was used.

In the molassed silage and A.I.V. fodder series there were five direct comparisons made under identical conditions. The total acetic and butyric acid values are summarized below, together with the average *pH* values.

Table XV. *pH, acetic acid and butyric acid contents of molassed silage and A.I.V. fodder (stated as percentages of the dry matter)*

	<i>pH</i>	Acetic acid	Butyric acid
Molassed silage	4.17	2.28	0.98
A.I.V. fodder	3.77	1.13	0.96

The figures are stated on a dry-matter basis, to make a direct comparison possible. The difference between the two types of silage lies entirely in the acetic acid content.

## DIGESTIBLE NUTRIENTS

It is possible to calculate the digestible nutrients in the fresh crops and in the different types of silage from the data in Tables XII and XIII; the results are tabulated below.

Table XVI. *Digestible nutrients in fresh grass and different types of silage made therefrom (stated as percentages of the dry matter)*

Type ...	Ordinary		Molassed		A.I.V.		Whey		Miscellaneous acid treatments	
	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage	Grass	Silage
Starch equivalent	61.6	48.7	63.7	56.9	62.6	55.5	60.2	58.4	61.1	52.1
Starch equivalent (corrected)	—	55.6	—	61.2	—	59.3	—	62.0	—	56.9
Protein equivalent	10.27	8.03	8.29	6.56	10.04	9.47	6.44	6.56	10.86	9.78
Protein equivalent (corrected)	—	11.48	—	8.86	—	11.50	—	8.44	—	12.34
Digestible crude protein	11.10	12.39	9.15	9.82	10.98	12.58	7.10	9.32	11.67	13.26
Digestible "true" protein	9.43	3.66	7.42	3.30	9.11	6.36	5.78	3.81	10.05	6.29

The results are very interesting. The starch equivalent values of all the fresh crops show a close agreement. The silages show variable values, the ordinary being the lowest, followed by the miscellaneous acid treatments. The other three types do not show any marked change in starch equivalent, and this is more marked when the corrected starch equivalent value is examined, all three showing values close to those of the fresh crop.

The protein equivalent values for the fresh crops show considerable differences, and the silages all show lower values than the corresponding fresh crop. The A.I.V. process is the most satisfactory in this regard. The silage made with added whey had the disadvantage of added protein and of being made only in small containers, which accounts for the anomaly of increased protein equivalent value, particularly in the corrected value. The corrected values, in which no penalty is laid on breakdown, show in all cases that the silages have been of equal value to the fresh crop in protein content. The higher values are due to the fact that has already been stressed, the arithmetical increase in protein figures stated as percentages resultant on losses of nitrogen-free extractives. The figures for digestible crude protein all show this same feature, and would indicate that the silages are equal in this respect to the fresh grass.

It is in the digestible "true" protein values that the reason for the better agreement in protein equivalent in fresh crop and silage made by the A.I.V. process is evident.

The addition of acid has throughout resulted in a product of higher "true" protein content, showing that the breakdown of the protein is controlled by such treatment, though it is not prevented entirely.

## NUTRITIVE LOSSES

The most important question in connexion with any conservation process is that of relative losses of nutrients. The figures of greatest importance are those of dry matter, digestible crude protein, starch equivalent and protein equivalent. The values for the individual silages are given below, together with the crude protein content of the samples and their pH values.

The first point worthy of notice is that the losses are not necessarily correlated with the pH values, nor the protein content.

## DRY MATTER

The loss of dry matter in the ordinary silage is of a low order, and does not differ much on the average from any other type. It is interesting to note that good silage with relatively low losses of dry matter has been made from material of high protein content. It is difficult in this type of silage to get the pH down below 4.5. The average loss of dry matter in the molassed silage is the lowest of all, but this is due to the inclusion of two silages made in small silos in which the losses were very low.

The silage made with added whey showed low dry-matter losses, as did the two types of acid silage.

In general, there was no appreciable difference in dry-matter losses between the various types of silage examined.

## STARCH EQUIVALENT

The starch equivalent is a better measure of feeding value than the dry matter. The ordinary silage shows the largest loss in starch equivalent, but all the others lie together some 5 per cent lower. The lowest is the silage made with added whey, but there were only four samples made in small silos. If silages 3 and 4 are left out of consideration in calculating the average values for the starch equivalent losses in the molassed silage, the figures become 29.7 and 24.3 per cent for the starch equivalent and corrected starch equivalent respectively. This makes the figures for the losses lowest in the A.I.V. fodder. Using the corrected starch equivalent losses brings nearly all the silages into line, the lowest values being for the A.I.V. fodder and silage made with added whey.

## PROTEIN EQUIVALENT

It is in this regard that the A.I.V. fodder shows up to the greatest advantage, although the values for whey are low. Excluding the values for the two small pit silages made with added molasses increases the

Table XVII. *The losses of dry matter, starch equivalent and protein equivalent, and the crude protein content and pH values of the various types of silage (gains shown with positive sign)*

Losses %								
No.	Crude protein	pH	Dry matter	Starch equivalent	Starch equivalent (corrected)	Protein equivalent	Protein equivalent (corrected)	Digestible crude protein
Ordinary silage								
1	13.74	4.10	19.9	30.5	27.9	13.7	1.5	2.5
2	13.99	—	17.4	32.4	24.7	35.1	1.1	1.0
3	17.24	4.87	23.9	49.8	42.9	62.1	45.2	45.2
4	17.85	4.57	15.1	21.1	12.0	20.3	+ 8.3	+ 8.3
5	20.12	4.80	13.0	34.2	20.4	30.3	+ 13.2	+ 13.1
6	20.22	—	21.5	38.2	28.5	38.1	17.5	17.4
7	20.27	4.92	16.6	40.3	24.7	36.0	+ 4.8	+ 4.7
Average	17.63	4.65	18.2	35.2	25.9	33.7	5.6	5.7
Silage made with added molasses								
1	11.30	4.16	19.9	31.1	28.0	34.3	14.2	13.2
2	11.53	3.96	21.1	26.0	22.6	28.3	7.0	6.9
3	12.56	3.73	7.3	10.6	3.2	14.5	+ 23.4	+ 22.3
4	13.02	4.07	5.9	18.3	11.7	22.7	+ 8.3	+ 8.3
5	16.27	4.09	21.0	33.6	28.2	38.6	20.9	21.0
6	16.57	4.11	22.2	33.5	27.6	43.2	23.7	23.5
7	17.12	4.51	15.2	24.1	15.1	30.1	1.8	3.1
Average	14.05	4.09	16.1	25.3	19.5	30.2	5.1	5.4
A.I.V. fodder*								
1	12.52	3.75	22.0	30.6	27.5	26.4	7.7	7.7
2	13.30	3.71	17.6	27.8	24.1	8.1	+ 13.7	+ 13.7
3	13.99	3.40	26.9	31.9	29.1	29.0	19.7	19.7
4	17.02	3.78	13.8	27.8	22.8	29.9	13.3	13.2
5	17.93	3.85	20.5	30.8	26.7	26.4	13.2	13.2
6	18.29	3.60	14.8	28.1	21.2	29.7	11.4	11.4
7	18.99	3.97	13.5	27.4	20.4	10.0	+ 12.4	+ 12.3
8	19.85	3.75	8.3	4.0	+ 3.2	+ 4.0	+ 24.3	+ 24.2
9	20.79	4.05	22.1	33.2	27.1	33.5	19.4	19.4
Average	16.96	3.76	17.7	26.8	21.7	21.0	3.8	3.8
Silage made with added whey								
1	12.57	3.66	16.0	19.0	15.0	26.0	+ 2.9	6.0
2	13.04	3.84	15.1	20.3	15.4	14.2	+ 10.7	+ 13.1
3	13.50	3.83	21.2	26.7	22.6	25.4	6.1	4.8
4	13.96	4.12	18.6	23.1	17.3	18.7	+ 9.3	+ 10.7
Average	13.27	3.86	17.7	22.3	17.6	21.1	+ 4.2	+ 3.2
Silage made with various acid treatments†								
1	13.53	4.30	13.6	21.4	15.9	25.1	5.5	5.5
2	16.61	4.11	18.3	30.3	25.2	28.5	12.4	12.4
3	18.33	4.19	20.5	37.1	28.7	28.8	1.5	1.4
4	19.30	3.96	22.0	34.4	26.1	30.0	3.5	3.4
5	19.41	3.80	14.3	27.0	20.6	16.8	+ 0.6	+ 0.5
6	20.41	3.40	19.5	33.9	29.2	30.0	18.5	18.5
Average	17.93	3.96	18.0	30.7	24.3	26.5	6.8	6.6
Av. 1-4	16.94	4.14	18.6	30.8	24.0	28.1	5.7	5.7

\* Another trial, where no digestibility trial was carried out, gave a loss of dry matter of 13.1 per cent.

† The treatments were: 1, Penthesta method ( $\text{PCl}_5$ , giving rise to  $\text{HCl}$  and  $\text{H}_3\text{PO}_4$ , together with sugar); 2, A.I.V. acid + sugar, to give pH 4.5; 3, Defu process,  $\text{HCl}$  together with phosphoric acid and sugar, to give pH 4.5; 4, Defu process using mono-ammonium phosphate in place of phosphoric acid; 5, 2N  $\text{H}_2\text{SO}_4$  added to give pH 3.5-4.0; 6,  $\text{POCl}_3$  dissolved in water, to give pH 3.5.

average for this type to 34.9 per cent. This is not unexpected, since experience has shown<sup>(1)</sup> that the breakdown of protein is greatest in silages where molasses is added, though this does not proceed beyond the stage of amino acids.

When allowance is made for the breakdown products of the "true" protein, the differences between the different silages disappear.

#### DIGESTIBLE CRUDE PROTEIN

The digestible crude protein of silage is probably the best criterion of the value of the nitrogenous constituents. It has been suggested that the non-protein nitrogen compounds in silage are of equal value to the "true" protein, from which they were formed, so long as the breakdown has not proceeded beyond the stage of amino acids, and recent work at this Station<sup>(16)</sup> has confirmed this.

The figures for digestible crude protein losses agree very well with the values for corrected protein equivalent and the differences between the various types of silage disappear. The losses of digestible crude protein are very low. In some cases gains of digestible crude protein were registered, seven of these in the small concrete pits. The accuracy of measurement here is not so great, since the amounts of crude protein in the silos are but small.

Silo 4 of the ordinary silage series and No. 8 in the A.I.V. series show increases in digestible crude protein which are attributable to low digestibility coefficients in the *fresh grass* going into the silo. It was fairly wet, and the sheep were loose on the diet, as a result of which digestion was upset, and the coefficients for the two silages showed increases over the fresh grass. This has given an advantage to the silage treatments which applies to all the digestible constituents and most to the crude protein. The average values show some slight advantage for the A.I.V. process over other types of silage, but it is not very marked.

#### ADDITION OF BACTERIAL CULTURES

The addition of bacterial cultures to some of the whey silages (Nos. 1, 3, 4) has made no difference at all to the losses. The least satisfactory treatment was Silo 3, in which inoculated dried whey was sprinkled on the mass, and therefore did not permeate the whole mass.

## COMPARATIVE TRIALS

In many cases direct comparisons were made between different processes, and these can be classed as follows in respect of the losses of dry matter.

Table XVIII. *Comparative losses of dry matter in various processes of silage making*

No. of comparisons	Size of silos	Loss of dry matter	
		Ordinary	v. Molassed
2	1 large	Ordinary (1, 4)	v. Molassed (4, 7)
	1 small	17.5%	10.6%
4	2 large	Ordinary (3, 4, 5, 7)	v. A.I.V. (9, 8, 7)*
	2 small	17.2%	14.4%
5	Large	Molassed (1, 2, 5, 6, 7)	v. A.I.V. (2, 1, 4, 5, 3)
		19.9%	16.4%
1	Large	Ordinary (4)	v. Molassed (7) v. A.I.V. (8)
		15.1%	15.2% 8.3%

\* Seven in A.I.V. series was control to 5 and 7 in ordinary series, and must be used twice in calculating the average.

The results show in general an advantage of the A.I.V. process over the others, but this is not of the order which might be expected from the literature. The comparisons were made in silos of similar size filled with grass from the same source, due precautions being taken to ensure evenness of filling. The comparisons have extended over a considerable period, and seem to show that the loss of dry matter is less by about 5 units (per cent) than the other processes, and that the molassed silage has lower losses of dry matter than well-made ordinary silage.

The most comprehensive series of comparisons was that made between molassed silage and A.I.V. silage. This was carried out in 1934 and 1935, one pair of silos being filled in the former year, and four pairs in 1935, two of concrete, two wooden. The treatments were assigned at random to the pairs of comparable silos, and grass of three different protein levels was used. The average values for this series are given in Table XIX.

The figures show the usual trends, there is an increase in the ether extract and in the non-protein nitrogenous compounds which is somewhat greater in the case of the molassed silage than the A.I.V. fodder. Since both are due to changes in the silo which are better controlled by the addition of acid, this was to be expected. There is a greater loss of crude and of digestible protein in the molassed silage, the difference being of the order of 13-15 per cent. The dry-matter and starch-equivalent losses show no great difference. Incidentally, it should be mentioned

that although the molasses solution was included in the calculations of losses, this was not done with the acid solution used in the A.I.V. process. This omission would affect the dry matter and ash, increasing the loss of the former to 18.2 per cent and changing the balance of ash, which was positive at +0.2, to a loss of 20.5 per cent of the total ash constituents in the fresh grass and the acid solution added to it. The relative significance of the differences between the A.I.V. fodder and the molassed silage is given below in terms of the most important constituents.

Table XIX. *Average losses in A.I.V. fodder and molassed silage. Five comparative trials in 1934 and 1935 (stated as percentages of the original grass). Gains shown with positive sign*

	Crude nutrients			Digestible nutrients		
	A.I.V. fodder %	Molassed silage %	Difference in favour of A.I.V. fodder	A.I.V. fodder %	Molassed silage %	Difference in favour of A.I.V. fodder
Dry matter	16.4	19.9	3.5	15.8	20.7	4.9
Ether extract	+72.8	+95.5	-22.7	+133.1	+184.4	-51.3
Fibre	3.5	1.7	-1.8	4.1	+2.3	-6.4
Crude protein	4.7	13.7	9.0	+0.8	13.5	14.3
Ash	+0.2	3.5	3.7	—	—	—
N-free extractives	32.1	37.4	5.3	32.5	38.7	6.2
Organic matter	17.9	21.5	3.6	16.6	21.1	4.5
"True" protein	30.8	44.5	13.7	40.0	61.4	21.4
Non-protein nitrogenous substances	+143.9	+151.6	-7.7	+165.4	+171.9	-6.5
Starch equivalent	—	—	—	24.2	29.7	5.5
Starch equivalent (corrected)	—	—	—	19.6	24.3	4.7
"True" protein (corrected)	—	—	—	+0.7	12.9	13.6
Protein equivalent	—	—	—	17.4	34.9	17.5
Protein equivalent (corrected)	—	—	—	+0.8	13.5	14.3

Table XX. *Standard error of differences in loss of nutrients of A.I.V. fodder and molassed silage*

	Difference mean	Standard error of difference	Significant difference ( $P=0.05$ )
Dry matter	3.44	1.57	4.35
Starch equivalent	5.46	4.05	11.24
Starch equivalent (corrected)	4.72	3.83	10.62
Protein equivalent	17.54	5.80	16.10
Protein equivalent (corrected)	14.28	5.52	15.32
Crude protein	8.98	1.80	5.00

While in all cases the A.I.V. fodder shows slightly lower losses than molassed silage, it is only in the case of the crude protein and protein

equivalent that it can be considered as significant. The position may be summarized by saying that the advantage of the A.I.V. process over molassed silage under our conditions is not likely to exceed 5 per cent of starch equivalent, 10 per cent of crude protein or 20 per cent of protein equivalent.

The comparison between the ordinary silage and A.I.V. fodder is not so straightforward, two of the comparisons being made on a large scale, two using small silos. The average figures are given below.

Table XXI. *Average losses in A.I.V. fodder and ordinary low-temperature silage comparative trials, 1932 and 1934 (gains shown as positive)*

	Four trials			Two trials in large silos		
	Ordinary (low-tem- perature) silage	A.I.V. fodder	Difference in favour of A.I.V.	Ordinary (low-tem- perature) silage	A.I.V. fodder	Difference in favour of A.I.V.
Dry matter	17.2	14.4	2.8	19.5	15.2	4.3
Starch equivalent	36.4	23.0	13.4	35.5	18.6	16.9
Starch equivalent (corrected)	25.0	16.2	8.8	27.5	12.0	15.5
Protein equivalent	37.2	9.9	27.3	41.2	14.8	26.4
Protein equivalent (corrected)	4.7	+7.4	12.1	18.5	+2.5	21.0

The A.I.V. process shows to greater advantage, although the dry matter losses do not differ by any greater amount than with molassed silage.

The starch equivalent values show a loss 13–17 per cent greater in the ordinary process, and the protein equivalent values show still wider divergences.

Whereas the A.I.V. process showed a 5 per cent advantage in starch equivalent over molassed silage, it is 15 to 20 per cent over ordinary silage, and in protein equivalent the advantage of 17 per cent over molassed rises to 27 per cent over ordinary silage, a figure which is not reduced much on correction.

## DISCUSSION

The main aim of this paper is to discuss the losses which occur in silage, as compared with the fresh grass, the chemical and bacteriological changes being discussed elsewhere (1, 20). The silages considered have all been made in simple containers, either of concrete or of wood. No great volume of work has been carried out on pit or stack silage, since it is obvious to anyone who has worked with such material that the dominant



factor is the degree of waste at the side and top. This varies markedly with the dimensions of the pit or stack, and it is therefore difficult to give a figure which may be regarded as being truly representative of pit or stack silage. That good silage can be made in a pit is undoubted, and if the pit be properly drained and the ingress of air controlled by a layer of soil, silage of excellent quality can be made by this means. The stack process is less easily controlled, and the losses may be exceedingly high, even though the material would appear to be well preserved and palatable. Such a case arises when the stack has heated considerably and the material has suffered heavy losses due to respiration. The digestibility of all the constituents is lowered in the process, and the material, though highly palatable and "sweet", may easily have lost 60 per cent or more of the original feeding value.

A stack of grass silage was made at Jealott's Hill in June 1932, using some 7 tons of grass. The stack was kept some months, the maximum temperature recorded being 130° F. It was all weighed out, sampled and analysed. The losses were high. Of the 4414 lb. of dry matter built into the stack, 1613 lb. of edible dry matter were recovered and 1243 lb. of waste. The total recovery was 2856 lb., a loss of 35.3 per cent without correcting for volatile constituents. The total loss, including inedible waste, was 63.5 per cent of dry matter. In addition, the digestibility of the "true" protein was reduced from 67.1 per cent in the fresh grass to 26.1 per cent in the silage, which would still further increase the losses of nutrients.

A second stack built in October 1931 and using 49 tons of grass was examined. It did not prove possible to weigh all the crop, but by sampling the area cut and later on cutting out, weighing and sampling a segment of the stack, it was estimated that the loss of dry matter was 34.1 per cent, but including waste, the figure rose to 50.6 per cent. The temperature had risen to 152–160° F., and in one spot to 165° F. The digestibility of the upper layers, which had heated most, showed coefficients of 24.8 per cent for the crude and 15.2 per cent for the "true" protein, whilst in the lower layer the values were 38.7 and 18.9 per cent respectively. The very heavy loss in protein due to over-heating alone is obvious when the usual level of digestibility of the protein of 70 per cent or more is used as a basis of comparison.

In October 1932 some silage was made by the low-temperature process *inside a portable silo built above ground*. When the material had settled, the wooden casing was removed. Though no data are available as to losses, the quality and digestibility of the product were found to be

excellent and to approximate to that of good tower silage. The silage which contained 19.43 per cent of crude protein in the dry matter had no waste, the sides having been properly consolidated in the case, and the earth cover having protected the top. It was all edible, and the digestibility of the organic matter was 68.3 per cent, the crude protein 68.0 per cent, and the "true" protein 52.4 per cent. It is clear from this that the silage was extremely good, and the use of portable silos is to be recommended where a less permanent structure is desired.

The only sample of pit silage examined was made in an earth pit 3 ft. deep, 14 ft. long, and 6 ft. wide. The pit held  $4\frac{1}{2}$  tons, heaped up to some 3 ft. above ground-level, and the maximum temperature was 124° F. The silage was of good quality and fair digestibility (organic matter 67.4 per cent, crude protein 54.2 per cent, and "true" protein 36.6 per cent).

Of the 2315.1 lb. of dry matter filled in, 1883.0 lb. were recovered (corrected for volatiles), from which must be deducted 299.1 lb. of waste. The loss due to waste was thus 13.1 per cent, but on a larger silo it would have been considerably less, and had the silo held 40 tons it would have been a negligible amount. The loss of nutrients suffered by the edible silage was 31.8 per cent of the dry matter, 49.0 per cent of the starch equivalent (45.5 per cent corrected) and 52.1 per cent of the protein equivalent, and 36.3 per cent of the digestible crude protein. Despite the relatively large amount of waste proportional to total weight, the losses are relatively high as compared with the figures obtained in the pit lined with wood or concrete which have been considered above.

In considering the losses obtained in this series of experiments, it must be realized in the first place that the type of crop used differs very considerably from that used in northern Europe, where the A.I.V. process is so popular. In this country the surplus produce obtained from grass-land in late May, early June, or again in the late autumn contains a preponderance of grasses very often in a leafy stage of growth. Such material packs easily in a silo, and with due care makes excellent silage. The main crop in northern Europe is a seeds mixture of Timothy and red clover, bulky, rich in protein, and difficult to pack so as to exclude air. Under similar conditions, using a grassy herbage, Drew *et al.* (21), Boyle & Ryan (22), Morley Davies *et al.* (23) and de Ruyter de Wildt *et al.* (24) have obtained results which agree closely with those put forward in this paper. These are summarized in Table XXII, together with our own values.

The general agreement between the losses of dry matter is obvious,

# 98      *Losses of Dry Matter and Nutrients in Silage*

the figures of Boyle & Ryan being considerably lower for the A.I.V. fodder than the others. The figures quoted by Drew and his co-workers for losses including waste are a truer measure of the practical value of the process.

Table XXII. *Losses of nutrients in ordinary silage and A.I.V. fodder (stated as percentages of fresh grass)*

	Ordinary silage	A.I.V. fodder	Molassed
Drew (21):			
Dry matter ... ..	17.30	12.60	—
Dry matter including waste ... ..	22.43	19.42	—
True protein broken down into simpler compounds	39.13	19.36	—
Boyle & Ryan (22):			
Dry matter ... ..	15.6	7.3	—
Davies <i>et al.</i> (23):			
Dry matter ... ..	24.1	18.1	12.5
Starch equivalent ... ..	36.2	12.8	24.0
Protein equivalent ... ..	53.3	15.0	42.5
Digestible crude protein ... ..	38.2	7.8	21.1
De Ruyter de Wildt <i>et al.</i> (24):			
Dry matter ... ..	15.2	9.4	—
Starch equivalent I* ... ..	21.5	9.1	—
Starch equivalent II ... ..	18.4	10.9	—
Digestible true protein ... ..	84.2	28.1	—
Present investigations:			
Dry matter ... ..	18.2	17.7	16.1
Starch equivalent ... ..	35.2	26.8	25.3
Protein equivalent ... ..	33.7	21.0	30.2
Digestible crude protein ... ..	5.7	3.8	5.4

\* Calculated on "true" protein plus "amides" (corrected value).

The loss of starch equivalent for A.I.V. fodder given by Davies *et al.* is surprisingly low in view of the figure for dry matter, but there was some doubt about the digestibility coefficients. In one case at Jealott's Hill (silage 8, A.I.V. series, Table XVII) the same effect was noticed, and here the apparent digestibility of the silage was definitely higher than that of the fresh grass, owing to the laxativeness of the latter food, which had to be fed when it was fairly wet.

A similar, though less marked, effect is seen in the results quoted by de Ruyter de Wildt and his co-workers.

The molassed silage shows up better than ordinary silage. The Dutch silage was made in the clamp by the warm fermentation process, and shows a marked breakdown in true protein as a result. The general trend of all the results is similar, and shows that the advantage in dry matter and starch equivalent losses of the A.I.V. process over ordinary or molassed silage is not so great as it is usually thought to be. The breakdown of "true" protein is reduced to a marked extent by the addition

of acid, but so long as the decomposition has not proceeded too far, this, as has been pointed out<sup>(16)</sup>, is no great disadvantage.

A considerable volume of information is available dealing with losses in different silage processes, but for this discussion it is only intended to consider those experiments in which a direct comparison of two or more different types has been made.

It is also thought advisable to leave out the results already mentioned of Golf & Gneist<sup>(9)</sup>, in which special silos were used and where using clover treated with sugar, with acid, and no treatment, the losses were negligible except for a 10 per cent loss of nitrogen-free extractives in the control treatment.

The conditions in these steel cylindrical silos of small capacity are such that drainage is impossible, and exclusion of air is very complete.

Fingerling & Ebert<sup>(25)</sup>, working with red clover, measured the carbon dioxide given off by different types of silage made in steel cylinders which held 70–75 kg. of fodder. From this was calculated the loss of nitrogen-free extractives. The control cylinders (no addition) gave values of 17.56 and 16.83 per cent. Adding hydrochloric acid and sugar resulted in lower values of 7.55 and 9.42 per cent, whilst hydrochloric acid alone gave 10.13 and 10.98 per cent, and sugar alone 14.75 per cent. Though these figures do not give the complete story, they are indicative, since the main losses fall on the nitrogen-free extractives.

On a practical scale, comparative trials have been carried out by Edin *et al.*<sup>(26)</sup>, and on a laboratory scale by Steiner<sup>(4)</sup>, working in Wiegner's laboratory. The main loss figures have been extracted from the results, and are summarized below. The crop used in both cases was a clover-grass mixture of a short ley type.

Table XXIII. *Losses of nutrients in silage made from clover-grass mixtures by different processes (as percentage of fresh crop)*

	Dry matter	Starch equivalent	Digestible crude protein
Edin <i>et al.</i> (26):			
Ordinary silage ... ..	—	23.5	—
Molassed silage ... ..	—	22.0	—
With HCl to pH 4.5 + sugar ... ..	—	33.0	—
With HCl to pH 4.0 ... ..	—	30.0	—
Steiner (4):			
May 1933: Large A.I.V. silo ... ..	5.72	3.15	15.90
Laboratory silo, A.I.V. ... ..	14.31	14.89	24.90
Laboratory silo, Ordinary ... ..	30.01	40.80	55.68
October 1933: Large A.I.V. silo ... ..	9.97	7.64	3.44
Laboratory silo, A.I.V. ... ..	16.28	22.74	10.87
Laboratory silo, Ordinary ... ..	27.85	45.45	31.03

The losses of starch equivalent shown by the Swedish workers agree tolerably well with those of the present investigation, despite the difference in the crop. The addition of acid has not improved the silage in so far as losses of starch equivalent are concerned, and examination of the results shows that the ordinary molassed and acid-treated silages were all of the same order of digestibility and of similar composition. In their summary the Swedish workers state that "the results showed that ordinary clover aftermath, which was not too coarse-stemmed, contains so much carbohydrate available for the production of organic acids that good silage can be made if the forage is thoroughly compacted and well tramped in airtight silos. In such a case there is but little improvement in quality to be expected from adding acids or sugar to the forage when ensiling".

The results of Steiner, on the other hand, show a much more favourable result from the addition of acid and have been widely quoted by Wiegner<sup>(27)</sup>. The comparative tests really comprise only the laboratory silos, but the figures for two large silos filled in May and October 1933 respectively are included as showing the lowest values on record for nutritive losses. A curious feature of these figures is the lower loss of starch equivalent than of dry matter. Since any losses would take toll of the most highly digestible portion of the crop, it is to be expected that the starch equivalent losses will normally be greater than those of dry matter, as indeed they are in all the other cases.

The extremely low dry-matter content of the material used (12·10 per cent in May, 13·34 per cent in October) has already been stressed. (Edin *et al.*<sup>(26)</sup> state that fresh aftermath clover contains 12·5–14·0 of dry matter when *wet with rain*, 14–15 where the dew is on the green fodder, and where dry on the surface 16–18 per cent.) No one with any knowledge of silage making would attempt to make ordinary silage from such material and expect to get a product of high quality, especially in a small container.

Under these conditions, the better results with added acid are understandable, since the material settled quickly and was very compact, thus reducing respiration losses due to rapid production of anaerobic conditions. The added acid would also check butyric acid formation, but in the ordinary silage it was marked, as might have been expected.

A certain amount of information is also available on losses incurred in processes of acid silage other than the A.I.V. process. Brouwer and de Ruyter de Wildt in Holland<sup>(24, 28)</sup>, using the Defu process, have compared it with the Dutch clamp process on grass. These can be

compared with the average value for Nos. 1-4 of the miscellaneous acid treatments in Table XVII.

Table XXIV. *Losses of nutrients in grass silage made by miscellaneous acid treatments*

	Ordinary silage	Acid treat- ment silage
Brouwer <i>et al.</i> (28) 1932:		
Dry matter ... ..	17.3	10.8
Starch equivalent I ... ..	23.9	10.7
Starch equivalent II ... ..	25.9	11.2
Digestible "true" protein ... ..	83.1	37.5
de Ruyter de Wildt <i>et al.</i> (24) 1933:		
Dry matter ... ..	16.8	18.3
Starch equivalent I ... ..	20.9	20.2
Starch equivalent II ... ..	23.4	22.0
Digestible "true" protein ... ..	85.1	39.1
Present investigation:		
Dry matter ... ..	18.2	18.6
Starch equivalent II ... ..	35.2	30.7
Starch equivalent I (corrected) ... ..	25.9	24.3
Protein equivalent ... ..	33.7	26.5

*Note.* The Dutch workers give two starch equivalents, II calculated on digestible "true" protein, I giving due allowance to non-protein nitrogen.

The smaller scale trial in 1932 shows bigger differences between the ordinary process and the acid process than the larger scale trial in 1933. As with our own experiments, the difference between the acid process and the ordinary process is small in dry matter and starch equivalent, but the breakdown of protein makes a marked difference in the values for losses of digestible "true" protein.

In 1934 Kirsch *et al.* (29) tested the Defu process on a small scale (200 kg. lots) on clover, and compared it with sugar and with whey.

Table XXV. *Losses in digestible crude protein and starch equivalent (gains as positive sign)*

	Digestible crude protein equivalent*	Starch equivalent*
Sugar added (Kirsch <i>et al.</i> ) ... ..	8.7	17.9
Molasses added (Present investigation) ... ..	5.4	19.5
Defu process (Kirsch) ... ..	13.4	12.6
Miscellaneous acids added (Present investigation) ... ..	5.7	24.0
Whey added (Kirsch) ... ..	21.0	21.9
Protein-free whey added with bacterial culture (Kirsch) ... ..	19.3	19.8
Protein-free whey added alone (Kirsch) ... ..	23.0	22.6
Whey added (Present investigation) ... ..	+4.2	17.6
Formic and hydrochloric acid (Alfasil) (Kirsch) ... ..	20.2	27.2

\* Allowance made for non-protein nitrogen (corrected value).

The results are interesting, although they cannot be regarded as being on a practical scale. The sugar or molasses treatments of the German

workers and ourselves agree well. The addition of acid and sugar in the Defu process has not had a marked effect on losses, though those of starch equivalent are lower with the German workers, and only half of those found in our series. The addition of whey has not proved satisfactory, whereas in our case using larger weights of grass (1 ton) the results were very satisfactory. The treatment using Alfasil, which is a mixture of formic and hydrochloric acids, is included to show the inefficiency of the process, which aims at sterilization of the fodder.

Finally, we would refer to an experiment described by Rehm(30), in which lucerne was conserved by the cold fermentation process developed at Tschechnitz, in which a special tight-fitting lid is used for the silo. The carbon dioxide resultant on respiration is kept in, and checks the changes in the silo. This process was compared with the Defu process, using Penthesta (phosphorus pentachloride) as a medium for acidification.

For the ordinary process a brick and a metal silo were used, but the latter only will be considered, since the Penthesta was also used in a metal silo. The control lucerne was chaffed, that acidified was left uncut. The losses were as follows (Table XXVI):

Table XXVI. *Losses of nutrients in lucerne silage made by the Tschechnitz process and with added Penthesta*

	Ordinary treatment	Acid treatment
Dry matter	12.0	36.8
Starch equivalent	1.3	39.8
Digestible crude protein	4.1	32.7

The results would appear to be in favour of the ordinary treatment, but the acidified silage was found to be very mouldy, and this raised the losses. This is unnecessary, and results obtained on grass at Jealott's Hill with Penthesta (No. 1 Miscellaneous Acid Treatments, Table XVII) show that the losses can be kept at a low level.

In addition to the work in which direct comparisons have been made between the different processes of making silage, there is a very large volume of information on the nutrient losses of the different types of silage which need not be mentioned here. It is, however, of interest to refer to some of the more frequently quoted values for the improved processes of silage making.

Poijärvi(31) in 1932 published the results of an investigation into the losses involved in the Virtanen (A.I.V.) process. Ten samples were examined by the bag technique on different crops, mostly mixtures of

clover and Timothy, which gave losses which have been summarized below. A further series of nine experiments were carried out with similar crops in 1934 (32), and are also included in the table.

In 1935 Spildo (33) measured the losses involved in the A.I.V. process, using small silos containing 4550 and 3510 kg. respectively of a Timothy-clover mixture (15 per cent clover) and a clover aftermath (40 per cent white clover with Timothy and other grasses).

Table XXVII. *Losses of crude constituents in the A.I.V. process, and cold fermentation process (stated as percentages of the fresh crop). Gains shown with a positive sign*

	Poijärvi (31, 32)		Spildo (33)		Cold fermentation (Kirsch & Hildebrandt) (34)
	1932 (average 10 values)	1934 (average 9 values)	Grass-clover mixture	After-math	
Dry matter	10.8	—	6.1	3.1	4.94
Ether extract	+ 50.2	—	+ 18.1	+ 119.7	+ 30.2
Fibre	0.8	0.6	5.2	4.7	1.88
Crude protein	9.5	6.2	8.2	1.6	± 0.0
Ash	7.3	6.1	12.1	18.6	3.32
N-free extractives	19.0	13.9	6.8	6.0	10.78
Organic matter	10.8	8.8	5.5	0.9	5.11
"True" protein	28.6	27.2	32.0	38.0	50.54
Non-protein nitrogenous substances	+ 108.5	+ 129.6	+ 81.2	+ 106.4	+ 428.6

Figures quoted by Kirsch & Hildebrandt (34) from the work of Völtz on cold-fermentation silage are also included in the table.

The figures obtained by Spildo show very small losses of the different constituents, but indicate that there had been an appreciable breakdown of the "true" protein. They represent ideal conditions of making, but the figures for cold fermentation silage made from clover in East Prussia without any addition, though the material was chaffed, are every bit as favourable except that the protein breakdown is still greater.

The figures obtained by Poijärvi in Finland show losses of a somewhat higher order, and if due allowance is made for the fact that these were determined by the use of weighed bags with its resultant lower figures, these show losses of a somewhat higher order than would be expected from the claims of the originator of the process (2), and in good agreement with our own experience. Reed (35) has also very briefly reported work with alfalfa, in which the addition of a mixture of hydrochloric and sulphuric acids (6 per cent by weight of a 2*N* solution) did not lessen the loss of dry matter as compared with silage made without any acid addition.



## CONCLUSIONS

It is obvious from a survey of all the comparative data that the ordinary process of making silage, properly applied, does not result in large losses, even where material of fairly high protein content is used. The addition of a readily fermentable carbohydrate, such as molasses, makes the result more certain, and gives a better quality of silage<sup>(1)</sup>, and is, for all practical purposes, the ideal method.

The addition of mineral acid gives a silage of good quality, and is an aid to control of the fermentation process. Where acid is added according to Virtanen's instructions<sup>(2)</sup> to bring the acidity of the mass rapidly to pH 3.0-4.0, the best quality of silage is made, and nutritive losses are lowest. It is clear from Table XX, however, that the advantage over molassed silage is of a low order, except in regard to protein, which is penalized by breakdown. The loss of crude protein is of the order of 10 per cent, which is not serious, and in view of the high feeding value of the non-protein nitrogen<sup>(16)</sup> the digestible crude protein is probably the best criterion for evaluating the nitrogenous compounds.

It must be remembered that the experiments described have been carried out on grassland herbage from permanent pastures and not from young "seeds" mixtures with large amounts of clover, such as are commonly used in northern Europe. In order to get the best results from these by the ordinary process, it has been usual to chaff the crop, in order that it may pack properly. For conditions in Great Britain it is clear that the molasses process cannot economically be replaced by any process using mineral acids, such as the A.I.V. process, since the margin allowed by the difference in losses between them is too small to allow of the greater expenditure on the acid solution, apart altogether from the relatively greater simplicity and safety of the molasses process.

Where silage crops are used in Great Britain, they are usually cut at such a stage of growth that they contain adequate carbohydrates, and the losses, according to Woodman & Amos<sup>(36)</sup>, are so low that they compare favourably with figures obtained in this series of experiments. The losses in the tower silo are put by them at under 10 per cent of the dry matter of the fresh crop if ensiled at the correct moisture content, though under certain conditions the losses may be higher.

With grass silage, the general recommendation can be made that no addition is necessary if the crop is at a fairly advanced stage of growth, but if cut whilst relatively young it is better to add molasses at the rate

of 15-30 lb. per ton, rising with the crude protein content, in order to ensure that the fermentation follows a suitable course.

Exclusion of air and stimulation of lactic acid fermentation are the guiding principles, and the lower layers should not be filled in too rapidly, or a "sour" type of silage rich in butyric acid will result.

### SUMMARY

A series of experiments designed to measure the losses in dry matter and nutrients in different types of silage are described. These extend over a period of 4 years. The ordinary or low-temperature process (maximum temperature 80-100° F.) is described either with or without added molasses or whey solutions, and is compared with processes in which solutions of mineral acids are added. The A.I.V. process of Virtanen, in which the acidity of the mass is reduced rapidly to pH 3.0-4.0 by the addition of a mineral acid solution, is considered in detail.

The methods of measuring the losses in silage making are discussed, and it is suggested that for best results no less than 8-10 tons should be made. This should be weighed carefully in and out of the silo, each load being sampled. Emptying of the silo should not take more than one or two days. The sampling of fresh crop and silage for dry-matter determination is examined statistically. The average losses of a number of trials are summarized in Table XXVIII.

Table XXVIII. *Losses of dry matter and nutrients incurred in the making of silage by different processes*

Type	Ordinary		Molassed		A.I.V.		Whey		Miscellaneous acid treatments	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Dry matter	18.2	13.0-23.9	16.1	5.9-22.2	17.7	8.3-26.9	17.7	15.1-21.2	18.0	13.6-22.0
Starch equivalent	35.2	21.1-49.8	25.3	10.6-33.6	26.8	4.0-33.2	22.3	19.0-26.7	30.7	21.4-37.1
Starch equivalent (corrected)	25.9	12.0-42.9	19.5	3.2-28.2	21.7	+3.2-29.1	17.6	15.0-22.6	24.3	15.9-29.2
Protein equivalent	33.7	13.7-62.1	30.2	14.5-43.2	21.0	+4.0-33.5	21.1	14.2-26.0	26.5	16.8-30.0
Protein equivalent (corrected)	5.6	+13.2-45.2	5.1	+23.4-23.7	3.8	+24.3-19.7	+4.2	+10.7-6.1	6.8	+0.6-18.5
Digestible crude protein	5.7	+13.1-45.2	5.4	+23.3-23.5	3.8	+24.2-19.7	+3.2	+13.1-4.8	6.6	+0.5-18.5
Number of trials	7		7		9		4		6	

These results are examined in the light of results of trials in the literature.

From the losses obtained in directly comparable trials, it is concluded

that although the A.I.V. process gives silage with the lowest losses, the advantage over the molasses process is not sufficient to justify the general application of the former. With material at an advanced stage of maturity, the ordinary low temperature process is quite adequate.

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# THE CHARACTER OF BARLEY GROWN ON SOIL MADE ACID WITH SULPHATE OF AMMONIA

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(With Plate II)

THE effect of sulphate of ammonia in tending to make soil acid when it is applied as a manure has been well known ever since about 1891 when Wheeler<sup>(1)</sup> first observed the phenomenon at the Rhode Island Experiment Station. Similar results to those observed by Wheeler have been found all over the world where this manure has been applied for many years on soils containing very little calcium carbonate. One of these cases has occurred at the Woburn Experimental Station, where acidity has developed which has been fatal to continued cultivation of both wheat and barley. Seeing that the soil in the continuous barley experiments at this station has been treated with sulphate of ammonia for fifty years, with or without other mineral manures, the conditions have given an excellent opportunity to study the effect of soils made acid in this way on the growth and composition of the barley plant.

The original soil which forms the basis of the action of sulphate of ammonia had the composition shown in Table I when the experiments were started in 1877, the figures being based on extraction with strong hydrochloric acid (2).

Table I. *Analyses of soil of Stackyard Field, Woburn  
Experimental Station*

	1st depth (9 in.) %	2nd depth (9 in.) %
Organic matter and loss on heating (containing nitrogen)	4.13 (0.166)	2.43 (0.094)
Oxide of iron	2.93	2.57
Alumina	3.61	2.84
Lime (CaO)	0.31	0.20
Magnesia (MgO)	0.14	0.16
Potash (K <sub>2</sub> O)	0.29	0.23
Soda (Na <sub>2</sub> O)	0.14	0.22
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.16	0.11
Sulphuric acid (SO <sub>3</sub> )	0.03	0.02
Insoluble silicates and sand	88.25	91.20
	<hr/> 100.00	<hr/> 100.00

There is no record of the acidity of the soil at the time when the experiment was commenced, though a recent measurement on a sample taken at that time gave a pH value of 6.1. After barley had been grown for fourteen years, the plots treated with 50 lb. of ammonia each year, in the form of sulphate of ammonia, began to show signs of barley failure<sup>(3)</sup>. This became worse and worse, and in 1898 it was found that the soil of these plots had become acid to litmus. The treatment with sulphate of ammonia was, however, continued, and barley was sown each year on the land. In 1897 and on several more recent dates, a portion of the plots which had had sulphate of ammonia was treated with 2 tons of lime per acre, but otherwise the treatment was continued. At the end of fifty years, in 1926, the addition of manures of every kind ceased, and a two-year fallow was taken, after which barley was again grown each year for the next five years without any further manuring.

In the period which followed the fifty years of manurial treatment, soils have thus existed which represent the resultant of the application of the manures, without any additional effect of further addition of manures. We are thus able to judge the final effect of the treatments on the soil and on the barley grown upon it, without any complication due to the fresh addition of the manurial materials.

In the present paper it is proposed to deal with the character of the barley grown not only on the acid plots, but, for comparison, with that on a number of other plots whose treatment had been different for the period of fifty years. The plots considered and their annual treatments per acre from 1877 to 1926 were as follows:

Plots 1, 7. No manure of any kind from 1877.

Plot 2a. Sulphate of ammonia, equal to 50 lb. ammonia till 1906 and to 25 lb. ammonia 1906-26.

Plot 2b. Sulphate of ammonia as on Plot 2a, but with 2 tons of lime in 1897, and also in 1912.

Plot 3b. Nitrate of soda equal to 50 lb. ammonia from 1877 to 1906 and to 25 lb. ammonia 1906-26.

Plot 4a. Mineral manures only, containing, from 1877 to 1906, 3½ cwt. superphosphate, 200 lb. sulphate of potash, 100 lb. sulphate of magnesia and 100 lb. sulphate of soda. After 1906, only 3 cwt. superphosphate and 56 lb. sulphate of potash have been annually applied.

Plot 5a. Mineral manures as on Plot 4a, and sulphate of ammonia as on Plot 2a.

Plot 5b. Mineral manures and sulphate of ammonia as on Plot 5a, but with 2 tons of lime in 1897, and also in 1912.

Plot 6. Mineral manures as on Plot 4a, and nitrate of soda as on Plot 3a.

Plot 11b. Farmyard manure, equal to 200 lb. ammonia from 1877 to 1906, and equal to 100 lb. ammonia from 1907 to 1926.

When the end of the fifty-year period was approaching, and also after its completion, the pH value of the soil with each of the above treatments was taken and the figures obtained are shown in Table II.

Table II. *pH values of soil and subsoil in water*

Plots	1922 Topsoil 0-9 in.	1927		1932 Topsoil 0-9 in.
		Topsoil 0-9 in.	Subsoil 9-18 in.	
1, 7	5.6	5.4	6.2	5.5
2a	4.1	4.5	5.5	4.4
2b	6.2	5.8	6.4	5.8
3b	6.0	5.8	5.9	5.6
4a	5.6	6.0	5.2	5.4
5a	4.4	4.8	5.4	4.7
5b	6.6	6.1	6.6	5.9
6	6.1	5.8	6.3	5.8
11b	6.1	5.8	6.0	5.8

The soils that have been treated with sulphate of ammonia, either alone (Plot 2a) or with mineral manures (Plot 5a), have had a *pH* value in all the years of below 5.1, the mineral manures added in combination with sulphate of ammonia have had a limited effect in preventing the acidification of the soils, the addition of lime in 1897 and 1912 to a total amount of 4 tons per acre, have kept the *pH* value from falling below 5.8, and the plots treated with nitrate of soda or with farmyard manure have retained, with the unmanured plots, a *pH* value of from 5.6 to 5.8.

Barley grown after a number of treatments which have not seriously affected the acidity of the soil can thus be compared with that produced on land with a *pH* value of 4.7 (Plot 5a in 1932) and of 4.4 (Plot 2a in 1932). The character of barley plants on the above plots was examined in the years between 1929 (immediately after a two years' fallow) and 1933, during which time the general luxuriance of the plants in all cases was diminishing (as none of the plots had any further manure added), but in which the general relative character of the plants was fairly well maintained.

#### I. PROPORTION OF BARLEY PLANTS THAT SURVIVE IN ACID SOILS

Many statements have been made that barley planted on such soils as those of Plots 2a and 5a above, dies soon after germination. This does not appear to be the case at any stage of the experiment. The acid plots were very much overrun with certain weeds, of which spurrey (*Spergula arvensis*) is the most abundant. This weed grew with special vigour and covered up completely the barley plants that had been produced. But if the spurrey were removed the barley plants were found to be present, if not as a full stand, yet as a very fair show of plants. The plants were, however, dwarfed, and while, as will be shown later, they in many cases completed their life cycle and produced grain, yet they rarely grew

beyond 8 or 9 in. in height, and were hence lost in and smothered by the growth of spurrey. The action is not, however, simply a smothering one, for similar dwarfed plants have been regularly obtained on these soils, where the weed herbage has been kept entirely under control.

At the same time, though the main effect of the acidity of the soil is to dwarf the plants of barley, the weaker plants do appear to die. The extent to which this death takes place on some of the plots mentioned above is shown in Table III, as the result of examinations made in 1931. The counts were made on plots sown at the same time and with the same drill, and consisted in measuring the number of plants per metre length of drill in each plot, with two varieties ("Plumage" and "Archer") of barley. The counts were made on 24 June, the crops having been sown on 19 March.

Table III. *Number of shoots per metre length of row*

Plots	pH value in 1932	Plumage variety	Archer variety
1	5.4	70.0	74.4
2a	4.4	36.0	22.7
2b	5.8	57.0	54.3
3b	5.6	56.7	82.0
4a	5.4	65.6	81.0
5a	4.7	50.0	45.3
5b	5.9	66.8	81.7
6	5.8	61.2	76.5
11b	5.8	55.3	94.7
Mean of plots with pH above 5.3		61.8 $\pm$ 5.7	77.8 $\pm$ 12.2

With both varieties of barley, there is a very substantial (and significant) reduction in the number of plants obtained on the more acid plots. On Plot 2a the reduction on the mean of the less acid plots is 41 per cent with the Plumage variety, and 71 per cent with the Archer variety. On Plot 5a the corresponding figures are 18 per cent with the Plumage variety and 41.5 per cent with the Archer variety.

These figures seem to indicate not only that plots as acid as 2a and 5a cause the death of many of the weaker plants, but also that some varieties are more susceptible to its influence than others. Thus the Archer variety seems very much more susceptible to the influence of the acid soil than is the Plumage variety.

The effect of an additional dose of sulphate of ammonia in causing a loss of plants is much greater with both varieties. This is shown by the results of manuring with sulphate of ammonia on adjoining acid and less acid plots. On both of the Plots 8a and 8aa sulphate of ammonia was added in two top dressings at the rate of 50 lb. ammonia per acre, the



sole difference being that the latter (8aa) had been limed in previous years and hence had a much higher *pH* value than 8a. Table IV shows the number of shoots per metre length in each case on 24 June.

Table IV. *Number of shoots per metre length of row*

Plots	<i>pH</i> value in water in 1932	Plumage variety	Archer variety
8a	4.7	14.0	11.3
8aa	5.8	71.7	89.7

The reduction in the number of shoots per metre length of row, on the acid plot, in presence of a new addition of sulphate of ammonia, has been as much as 80 per cent with the Plumage variety, and 87 per cent with the Archer variety.

It seems clear, therefore, that while in an acid soil without any further addition of sulphate of ammonia, there is a certain loss of plant due to the acidity, yet the greater part of the plants are not killed, but merely dwarfed. On the other hand, the actual additions of fresh amounts of sulphate of ammonia after barley is sown in a soil with a *pH* value of 4.7 is very fatal, and may reduce the number of shoots that survive per unit area by between 80 and 90 per cent.

## II. CHARACTER OF BARLEY PLANTS IN ACID SOILS

When barley seeds are germinated in the laboratory in such acid soil as we have described, with a *pH* value of 4.4–4.7, germination does not appear to be much delayed and is complete. In the field, however, the plots where the soil is acid always require a longer time for the plants to appear above ground. But, once above ground, it is at first impossible to distinguish the plants on the acid land from those on other plots in the same field. After a few days, when the plants have not more than four leaves, and often when they have not more than two leaves, the tips acquire an unhealthy yellow colour. The next stages are the development of a purple colour on the lower leaves and especially on the leaf sheaths, while the whole plants take on a very stiff appearance. These appearances rapidly become emphasized as the plants get older. Four weeks from sowing, in the case of barley on soil from Plot 2a (with *pH* value of about 4.4), it was noted that, though there was hardly a plant with more than three leaves, yet there was scarcely a leaf (apart from quite new leaves) which was not purple, particularly on the veins, or which had not a yellow tip. The new leaves at this stage were still





1

2

3

4

generally healthy in appearance, but, even among them, some of the leaf tips were yellow. The stems were purple in colour in every case.

Plate II shows the appearances after 83 days from sowing on Plots 2a and 5a in comparison with those on plots which are less acid. These show also the character of the roots, which are not merely twisted and deformed (as was noticed by Voelcker in 1901 (4)), but are very definitely thickened, and this thickening was noticed throughout the life of the plants.

By this stage (i.e. 83 days from sowing) the dwarfing of the plants on the more acid plots was very marked, but there is no very great reduction in the number of the leaves. The actual figures, in the field, at this stage in 1929 are shown in Table V. The plots are arranged in the order of pH value. In the same year similar observations were made a little later when the heads of the plants were well developed, and similar results were obtained. Measurements were also made 111 days after sowing, on 10 July after the fruiting heads were well developed.

Table V. *Characters of barley plants on soils of different acidity, 1929*

Plots	pH value of soil in 1932	Dry matter in fresh plants %	Mean height per plant cm.	No. of shoots per plant	No. of leaves per plant	Wt. 100 air-dry plants g.
83 days after sowing:						
2b	5.8	29.2	39.8	2.1	6.0	77.4
1	5.4	28.6	45.7	1.5	6.7	49.2
5a	4.7	37.5	42.2	1.0	6.7	19.9
2a	4.4	43.1	27.8	1.0	5.7	11.7
111 days after sowing:						
2b	5.8	37.5	47.8	2.1	7.84	116.9
1	5.4	37.4	46.0	1.4	7.96	97.2
5a	4.7	45.1	26.4	1.1	7.84	32.4
2a	4.4	46.5	22.1	1.0	7.94	18.6

Both these sets of figures show how extremely deficient in water are the plants on the acid plots and that the dryness is greater the greater the amount of acidity. The height figures show how much the plants on Plots 5a and 2a are dwarfed, but they have almost the same number of leaves on the main shoot of each plant as are found in the less acid plots. The life of the plants, in fact, seems to go on to its ordinary conclusion, but the parts are very much reduced in size. This is shown very markedly in the weight of the plants, which seem to show a complete breakdown in the ordinary metabolism of the plants when a certain degree of acidity—lying between a pH of 4.7 and 5.4—is reached in the field.

Similar results were obtained from plants grown on the same and also from other plots of the same series in 1931 and 1932. A summary of the figures from each of the plots mentioned on p. 109 is given in Table VI.

All the plots (seven in number) whose soil has a *pH* value of over 5.4 are placed together, and compared with the figures for the plots which have a *pH* value of 4.7 (Plot 5a) and of 4.4 (Plot 2a).

Table VI. *Characters of barley plants on soils of different acidity, 1931-2*

Plots	<i>pH</i> value of soil in 1932	Dry matter in fresh plants %	Mean height per plant cm.	No. of shoots per plant	No. of leaves per plant	Total heads per plant	Wt. 100 air-dry plants g.
1931. Time from sowing—103 days (30 June)							
Mean of 7 plots	Over 5.4	30.6 ±1.1	36.9 ±1.9	1.36 ±0.22	6.7 ±0.6	0.68 ±0.13	71.6 ±20.1
5a	4.7	42.4	18.0	1.03	6.4	0.17	14.5
2a	4.4	37.9	16.8	1.00	6.6	0.00	12.5
1932. Time from sowing—92 days (16 June)							
Mean of 7 plots	Over 5.4	33.6 ±0.9	28.9 ±3.4	1.50 ±0.34	6.1 ±0.2	No heads	45.3 ±17.1
5a	4.7	36.5	21.1	1.10	5.7	—	20.5
2a	4.4	38.6	16.3	1.00	5.7	—	13.2
1932. Time from sowing—139 days (23 July)							
Mean of 7 plots	Over 5.4	45.2 ±1.8	37.2 ±6.4	1.29 ±0.20	6.2 ±0.2	0.94 ±0.06	—
5a	4.7	43.4	25.1	1.00	5.8	0.85	—
2a	4.4	57.3	15.2	1.00	6.1	0.13	—

The last column, with the plants taken on 23 July 1932, can hardly be calculated merely as a function of the acidity, and hence I have divided the less acid plots into (1) those plots which never had nitrogenous manures during the previous fifty years, (2) those which had nitrogenous manures but no mineral manures in the previous fifty years, (3) those which had both nitrogenous manures and mineral manures during the previous fifty years, and (4) that which had farmyard manure in the previous fifty years. If this is done we get the following (Table VII):

Table VII

Plots	Mean <i>pH</i> value of soil	Treatment from 1877 to 1926	Mean wt. 100 air-dry plants g.
1, 4a	5.4	No N manures	55.3
2b, 3b	5.8-5.6	N manures only	61.2
5b, 6	5.9-5.8	N and mineral manures	116.7
11b	5.8	Farmyard manure	167.8
5a	4.7	N and mineral manures	31.0
2a	4.4	N manures only	8.4

How far do the same differences continue till the plants are ripe? Table VIII gives the figures, arranged in the same manner as in Table VI,

obtained from representative samples at the time of harvest in several years.

Table VIII

Plots	pH value of soil in 1932	Mean height of plants cm.	No. of shoots per plant	No. of heads per plant	Mean length of main head cm.	Mean no. of grains in main head	Wt. 100 air-dry plants g.
1929. Plants at harvest							
Mean of 5 plots	Over 5.4	56.0 ±6.5	1.85 ±0.38	1.17 ±0.10	4.5 ±0.4	15.7 ±1.9	185.0 ±49.0
5a	4.7	38.0	1.87	1.10	2.6	8.7	100.0
2a	4.4	19.2	1.15	0.79	1.2	1.2	13.5
1930. Plants at harvest							
Mean of 5 plots	Over 5.4	46.6 ±3.1	1.51 ±0.24	0.99 ±0.08	4.1 ±0.4	13.9 ±1.3	136.0 ±16.0
5a	4.7	23.9	1.67	0.65	1.9	1.6	30.0
2a	4.4	18.9	1.55	0.28	1.2	1.5	14.0
1931. Plants at harvest							
Mean of 7 plots	Over 5.4	43.7 ±8.0	1.40 ±0.27	1.03 ±0.09	3.6 ±0.1	13.2 ±4.8	140.0 ±75.0
5a	4.7	33.0	1.07	1.00	1.8	6.6	48.0
2a	4.4	25.4	1.03	0.92	1.3	2.9	25.0
1929, 1930, 1931. Mean of three years. Plants at harvest							
Mean of plots	Over 5.4	48.8	1.59	1.06	4.1	14.3	154.0
5a	4.7	31.6	1.54	0.92	2.1	5.6	59.0
2a	4.4	21.2	1.24	0.66	1.2	1.9	17.5

Apart entirely from the content of mineral manures, acidity clearly leads to a dwarfing of the barley plants, and this becomes evident at a pH value below 5.4. Above this, the question of the degree of acidity seems to make little difference either to the height or weight of the plants. Below this point, the dwarfing rapidly becomes greater as the degree of acidity is increased. There seems to be indications of a critical degree of acidity below which the normal life of the plants cannot take place. This critical point would vary with the conditions of culture, for Brenchley(5) has shown that in water culture barley will still give normal growth at a considerably higher degree of acidity than is found in Plot 5a above. But in the Woburn soil, under the conditions of field culture, the critical point seems to lie at a stage represented by an acidity slightly less than pH 5.0.

The most striking result of the injurious degree of acidity in these soils lies in its effect on the formation of ears and the grain contained in them. The length of the ears is at once reduced when the pH falls to 5.0 and decreases very rapidly at acidities greater than this. The

number of grains formed and ripened in the ears becomes even more rapidly reduced, and with the higher acidity represented in Table VIII ( $pH$  4.4) it is rare that the grains exceed two per head. On the other hand, the number of shoots per plant, and even the number of heads per plant, does not seem to be so much or so directly influenced by the acidity of the soil.

In other words, with an increase of acidity of the soil from  $pH$  5.4 to 4.4, the vegetative growth is reduced and the plants dwarfed, together with the colour changes which are the evidence of stress in the plant organism and which have been described above. All the same, the life history of the plant seems to go on to completion, but the ears become very much reduced in size and the number of grains that ripen tends to be very small.

### III. COMPOSITION OF BARLEY PLANTS IN ACID SOILS

The analysis of the plants was commenced in 1929, when barley was grown after a two years' fallow and, except on the acid plots, the growth was good and the crop quite a reasonable one. At first only plants from four selected plots were taken, but, later, produce from most of the plots referred to in the previous section were examined.

One of the first things noticed when plants grown on the acid plots were burnt was that the ash from barley plants on the acid soils was not white, but always showed signs of containing an excess of oxide of iron. This has been found throughout the investigation in samples taken from plants at every stage of development. The only exception to this is the case of the grain produced from such plants which did not show signs of containing a large excess of this constituent.

Apart from this question of the presence of excess of iron in plants grown on acid plots, the first examinations of growing plants, taken 83 days after sowing in 1929, gave the figures shown in Table IX, the plots being arranged in order of their  $pH$  value. The figures represent the analyses of the whole plants including the roots, and are based on the completely dry material.

Table IX. *Analyses of barley plants taken on 12 June 1929*

Plots	$pH$ value of soil in 1932	Ash, less sand and silica %	Lime (CaO) %	Phosphoric acid ( $P_2O_5$ ) %	Sulphuric acid ( $SO_3$ ) %	Nitrogen %
2b	5.8	4.23	0.31	0.664	0.698	1.14
1	5.4	5.34	?	0.412	0.499	1.34
5a	4.7	3.31	0.15	0.526	0.598	0.87
2a	4.4	2.84	0.26	0.320	0.499	1.00

In these plants, taken before the ears are formed and emerged, the ash constituents other than silica are decidedly low in the barley from the acid plots, the lime is low in amount, and the nitrogen is very much reduced. I have no further analyses of plants at such an early stage, but in 1931, on much inferior barley, I have analyses of plants taken on 30 June (103 days from sowing) and on 16 June 1932 (92 days from sowing). The full details are not shown, but a summary, in which all the analyses of barley from soils with pH value of over 5.4 are combined together, is given in Table X.

Table X. *Analyses of barley plants at 92-103 days after sowing*

Plots	pH value of soil in 1932	Ash %	Silica %	Oxide of iron %	Alumina %	Lime %	Phosphoric acid %	Nitrogen %
1931. Time from sowing—103 days (30 June)								
Mean of 7 plots	Over 5.4	5.48 ±0.21	1.46 ±0.13	0.111 ±0.029	0.090 ±0.020	0.485 ±0.080	0.632 ±0.044	0.88 ±0.06
5a	4.7	3.93	0.88	0.405	0.314	0.230	0.451	0.62
2a	4.4	3.84	1.06	0.490	0.326	0.197	0.350	0.69
1932. Time from sowing—92 days (16 June)								
Mean of 7 plots	Over 5.4	6.86 ±0.24	1.59 ±0.12	0.200 ±0.021	0.167 ±0.021	0.664 ±0.100	0.667 ±0.091	1.02 ±0.08
5a	4.7	5.53	1.00	0.416	0.245	0.407	0.755	0.91
2a	4.4	4.53	1.07	0.320	0.330	0.259	0.425	0.83
1931 and 1932. Mean of the above analyses								
Mean of 7 plots	Over 5.4	6.17	1.52	0.155	0.128	0.574	0.649	0.95
5a	4.7	4.73	0.94	0.410	0.279	0.318	0.603	0.76
2a	4.4	4.18	1.06	0.405	0.328	0.228	0.387	0.76

Now the most obvious fact about these figures is the very great constancy of the composition of barley plants grown on the same soil at the same time, except in the case of the acid plots. In these acid soils we have (1) a considerable reduction in the amount of ash, and also in the amount of silica, (2) a very large increase in the amount of oxide of iron and also of alumina, which amounts in some cases to three times the normal percentage of each constituent, (3) a considerable reduction in the content of lime, which naturally reflects the exhaustion of the soil in this constituent, (4) a lowering in the amount of phosphoric acid, and (6) a considerable lowering in the percentage of nitrogen in the plants.

These conclusions apply only to the young plants, as taken not later than 30 June when they may have flowered but the grains have not really begun to form. A set of determinations was made, however, after the grain was forming in the ears but had not filled or become anything



like ripe. I have such a set of analyses for the year 1932 (taken on 23 July), and these will show how far the differences in composition which have been shown at an earlier stage continue after the ears have formed. As before, the whole plants were analysed (Table XI).

Table XI. *Analyses of barley plants at 139 days from sowing*

Plots	pH value of soil in 1932	Ash %	Silica %	Oxide of iron %	Alumina %	Lime %	Phosphoric acid %	Nitrogen %
Mean of 7 plots	Over	4.65	1.62	0.137	0.117	0.402	—	0.78
5a	4.7	±0.31	±0.19	±0.039	±0.032	±0.046		±0.06
2a	4.4	4.71	1.37	0.357	0.211	0.296	0.602	0.87
		4.02	1.56	0.791	0.261	0.230	0.453	0.91

By the time that the ears are formed, the differences which are so obvious in the young plant are tending to disappear. The ash, the combined silica, and the nitrogen are not significantly different in the plants from the more acid plots from what is found in the plots which produce normal plants. The phosphoric acid is, likewise, not significantly different in the produce from the more acid plots, provided notice is taken of the difference between the plots which for fifty years have received phosphatic manures and those which have not received phosphatic manures at all. On the other hand, the plants from the more acid plots still contain very markedly higher amounts of iron and aluminium and markedly less lime than those which were grown on less acid soils.

How far do these differences still show at the time of harvest, and in what part of the ripened product are the differences most marked at that time? In 1929 the corn and straw were examined separately. As there seemed no appreciable difference in the constituents determined in the grain, the examination in succeeding years was confined to the straw.

The actual figures for the barley grain on which this statement is

Table XII. *Analyses of barley grain produced in 1929*

Plots	pH value of soil in 1932	Ash %	Silica %	Oxide of iron %	Alumina %	Lime %	Phosphoric acid %	Nitrogen %
11b	5.8	1.92	0.46	0.018	0.065	0.132	0.899	1.31
2b	5.8	2.00	0.27	0.014	0.057	0.103	0.773	1.33
3b	5.6	2.07	0.40	0.019	0.082	0.138	0.741	1.38
4a	5.4	2.43	0.53	0.011	0.060	0.152	0.792	1.27
1	5.4	2.06	0.28	—	0.067	0.105	0.785	1.42
5a	4.7	2.02	0.40	0.020	0.050	0.140	0.807	1.42
2a	4.4	1.89	0.30	0.013	0.070	0.124	0.781	1.38
Mean		2.06	0.38	0.016	0.064	0.128	0.797	1.36
		±0.18	±0.10	±0.004	±0.010	±0.018	±0.049	±0.05

based are shown in Table XII, the plots being arranged in order of the acidity of the soil.

There are no significant differences from the mean value in each case, with the possible exception of the ash on Plot 4a and the phosphoric acid on Plot 11b. Even in these cases the differences barely approach significance. There is certainly in no case any evidence that the composition of the grain has been, so far as the ash constituents and nitrogen are concerned, affected by the differences in acidity.

The case is quite different with the straw and, hence, the analysis of this material was carried on for the plots in question for three years, and Table XIII is a summary of the results.

Table XIII

Plots	pH value of soil in 1932	Ash %	Silica %	Oxide of iron %	Alumina %	Lime %	Phosphoric acid %	Nitrogen %
Barley straw produced in 1929								
Mean of 5 plots	Over 5.4	4.29 ±0.69	2.20 ±0.86	0.065 ±0.012	0.060 ±0.014	0.340 ±0.056	0.143 ±0.014	0.37 ±0.04
5a	4.7	2.37	1.16	0.112	0.088	0.249	0.214	0.45
2a	4.4	4.02	2.09	0.173	0.117	0.228	0.206	0.62
Barley straw produced in 1930								
Mean of 5 plots	Over 5.4	5.51 ±0.64	2.91 ±0.43	0.205 ±0.066	0.121 ±0.044	0.372 ±0.045	0.272 ±0.046	0.46 ±0.06
5a	4.7	5.74	2.29	0.446	0.319	0.380	0.364	0.86
2a	4.4	4.89	2.39	0.364	0.637	0.212	0.290	1.09
Barley straw produced in 1931								
Mean of 7 plots	Over 5.4	4.42 ±0.26	1.87 ±0.32	0.197 ±0.045	0.160 ±0.044	0.339 ±0.028	0.265 ±0.036	0.43 ±0.06
5a	4.7	3.24	1.02	0.222	0.179	0.326	0.287	0.49
2a	4.4	4.79	2.04	0.398	0.332	0.337	0.361	0.69

These analyses, taken from ripened plants from the selected plots, for three successive years, after the removal of the grain, tell a fairly consistent story. In the first place the amount of ash, and the silica contained in it, is apparently not affected by the acidity of the soil. Further, while the lime in the plants is markedly less in the straw from the acid plots than in the straw from the more normal plots, this is not the case in the last year when the general standard of luxuriance is considerably less. The excess of oxide of iron and of alumina continues on the acid plots throughout. The mean content of these constituents is less on the straw grown on plots which have had mineral manures, and least of all on the plot which had been treated with farmyard manure. There is definitely an accumulation of phosphoric acid in the straw on

the acid plots and still more markedly is there an accumulation of nitrogen.

The question at once arises, especially in view of the composition of the plants previously recorded, whether these variations in the straw on the acid plots are not merely due to the accumulations of materials which would normally be withdrawn to furnish the needs of the grain. Where little grain is formed, it would be natural that the excess in the straw would be rather a result of lack of the withdrawing power of the grain than of lack of absorption from the soil. I think that this is probably the explanation of the results here being apparently contrary to those found in the growing plant.

One more test has been made, namely the analysis of the roots of barley plants grown on these plots in 1929. The analyses in this case are not so reliable, partly owing to the difficulty in getting good samples of the complete root and partly on account of the difficulty of removing the whole of the attached soil. The results (Table XIV) only show one thing for certain, namely, that the roots of barley on the more acid plots contain a larger amount of nitrogen than is contained in the roots of the barley on the less acid plots.

Table XIV

Plots	pH value in 1932	Nitrogen %	Mean of 5 plots, 0.64 $\pm$ 0.02
11b	5.8	0.67	
2b	5.8	0.66	
3b	5.6	0.64	
4a	5.4	0.64	
1	5.4	0.61	
5a	4.7	0.85	
2a	4.4	1.19	

#### IV. GENERAL CONCLUSIONS

The results of this study of barley plants grown in soils acid enough to injure the plants and produce an abnormal growth seem to be fairly clear. Barley grows normally in a soil such as that used in the experiments reported at least until the acidity reaches a higher degree than that represented by a pH value of about 5.4. If the acidity is less than this, the character of the plants is very similar in all cases between a pH value of 5.9 and 5.4. This is the case whether the normality is judged by the vegetative growth, by the character of the earing, by the amount of grain, or by the composition of the plants during growth, or of the straw and corn at harvest.

When the degree of acidity of the soil under field conditions reaches a point certainly greater than pH 5.4 and probably less than 4.7 in the soil at Woburn, the normality of the plants seems to be destroyed. They are dwarfed, and the grain-bearing character is particularly interfered with. So far as the vegetative characters are concerned, such as the number of leaves formed, the number of shoots per plant, and the number of heads per plant, there is interference with the normal functioning of the plant, but, except for the dwarfing, this interference is not very great, though the plants show themselves sickly, the leaves tend to become yellow at the tips and purple in the veins, and the roots are thickened and twisted. But the grain-bearing power of the plants is very largely destroyed, though a few ears are actually formed with one or two grains in them. All these appearances are increased with increasing acidity beyond what we may call the "critical point".

In composition the plants grown in the soils with acidity lower than pH 5.4 are little affected. Here again, with acidity higher than the critical point, there are very substantial changes in composition at least in the early vegetative stages. In the more acid soils there appears to be a reduction of the ash of the plants, including the amount of silica in it; a very large increase in the amount of oxide of iron and alumina in the ash, which amounts in some cases to three times the normal amount; a reduction in the amount of lime, which naturally reflects the exhaustion of the soil in this constituent; a lowering in the amount of phosphoric acid, which is also reflected in the very great tendency of barley on acid soils to turn a purple colour (see Brenchley(5)); and a considerable reduction of the percentage of nitrogen in the plants.

These differences are very marked in the plants before ear formation begins. After this stage the differences become less clear, but the excess of oxide of iron and alumina, and the deficiency of lime are still marked in the plants from plots whose acidity is greater than the critical point.

At harvest the small amount of grain produced from the extremely acid plots does not differ in composition, so far as the constituents determined are concerned, from those on less acid soil. In the straw the excess of oxide of iron and alumina is still marked, but, owing to the non-formation of grain, the deficiency of lime has disappeared, and there is an actual accumulation of phosphoric acid and of nitrogen in the straw as against that found in straw produced on less acid soil. The roots of the plants on the acid plots contain markedly an increase in the amount of nitrogen.

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## EXPLANATION OF PLATE II

Barley plants 83 days from sowing.

1. No manure for 50 years. pH value of soil, 5.4.
2. Sulphate of ammonia for 50 years. pH value of soil, 4.4.
3. Sulphate of ammonia for 50 years and 4 tons of lime per acre. pH value of soil, 5.8.
4. Sulphate of ammonia for 50 years, with phosphates and potash. pH value of soil, 4.7.

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# IMPROVED TECHNIQUE IN GRADING OF COARSE AND FINE SANDS DURING MECHANICAL ANALYSIS OF SOILS

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(With One Text-figure)

IN the accompanying article attention is drawn to a source of error which arises in grading coarse and fine sand subsequent to dispersion. The method of mechanical analysis in which the error arises, and to which the writer refers, is the revised official method adopted several years ago by the Agricultural Education Association<sup>(1)</sup>. The usual procedure is to wash the dispersed soil on to a No. 70 I.M.M. sieve with a jet of hot water, and to rub it gently with a rubber pestle or the finger until no more will pass through. Robinson<sup>(2)</sup> states that this rubbing is generally unnecessary. The writer has found, however, that even prolonged washing and rubbing with a rubber pestle are ineffective in removing all the fine sand. In the wet mixture the sands are held together closely by surface tension. In order to overcome this, it would seem reasonable first to dry the sieve and its contents at 105° C. and give a further light sieving. This has been done with a large variety of soils, and it has been found that an extremely high proportion of fine sand is withheld when working with certain soils. The amount of fine sand sieved out of the dried mixture increases or decreases in proportion to the amount of total sands present. This is illustrated in the accompanying graph (Fig. 1), where it is to be noticed that, in soils increasing in total sands from 50 per cent (or thereabouts) upwards, the error begins to assume serious proportions. Hence soils most exposed to this error range from sandy loams to coarse sands, reaching maximum error in soils in which the ratio of true coarse sand to true fine sand is roughly 3 : 1. Table I shows the relation which the fine sand removed by dry sieving of the coarse sand fraction bears to the percentages determined by the official method.

The figures show considerable variation, and though, as in samples 13, 14 and 18 (soils containing over 60 per cent silt and clay), no appreciable

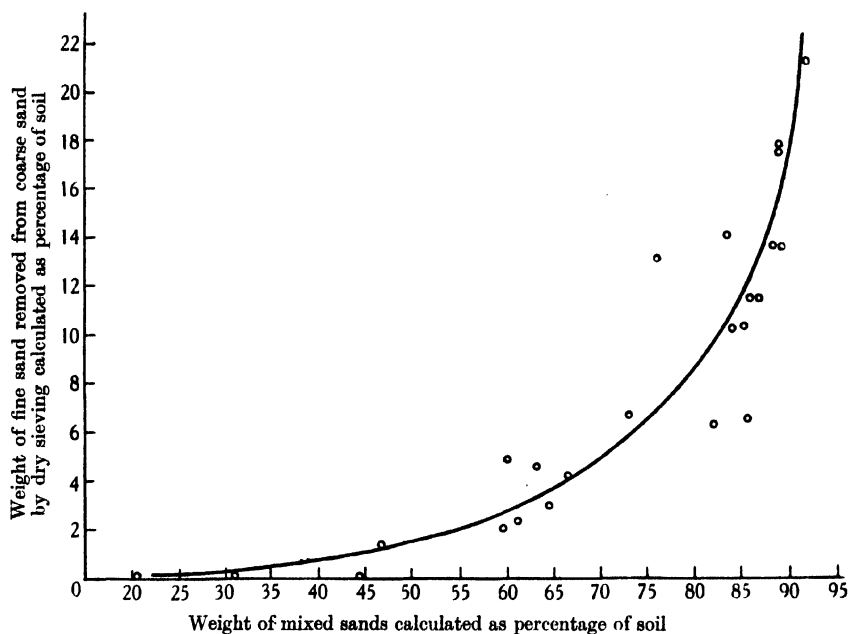


Fig. 1.

Table I

Fine sand dry sieved out of coarse sand and calculated as % of

Sample	Soil	Coarse sand and fine sand		
		Coarse sand	Coarse sand	Fine sand
1	10.3	12.0	25.8	22.5
2	14.0	16.7	31.3	35.7
3	3.0	4.6	13.4	7.0
4	2.4	4.0	10.8	6.1
5	11.4	13.3	18.2	49.4
6	13.6	15.3	20.0	65.6
7	4.0	6.0	21.1	8.4
8	4.6	7.2	23.8	10.2
9	10.2	12.1	16.5	46.4
10	13.0	17.1	23.3	63.4
11	6.5	7.6	10.0	31.2
12	12.7	14.2	17.2	80.6
13	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0
15	6.3	7.7	11.6	23.1
16	4.9	8.1	26.2	11.7
17	2.0	3.3	12.6	4.5
18	0.0	0.0	0.0	0.0
19	17.4	19.4	21.9	172.0
20	1.4	3.0	10.0	4.3
21	11.4	13.1	15.2	98.7
22	21.2	23.1	25.3	265.2
23	6.7	9.1	25.5	14.2
24	17.7	19.8	21.9	214.1

quantity of fine sand was removed by dry sieving, the instances in which dry sieving has produced highly positive results are far too frequent.

In Table II the above results are set out to show the deviation in composition resulting from the two methods of treatment. In column A the figures were obtained by the ordinary method of lightly pestling the sands. The corrected figures after dry sieving are shown in column B.

Table II

Coarse sand		Fine sand				Coarse sand		Fine sand	
Sample	A	B	A	B	Sample	A	B	A	B
1	39.8	29.5	45.7	56.0	13	4.8	4.8	15.4	15.4
2	44.6	30.6	39.2	53.2	14	8.9	8.9	21.8	21.8
3	22.4	19.4	42.5	45.5	15	54.4	48.1	27.5	33.8
4	22.2	19.8	38.9	41.3	16	18.5	13.6	41.4	46.3
5	62.8	51.4	23.2	34.6	17	15.8	13.9	43.9	45.8
6	68.0	54.4	20.7	34.3	18	14.2	14.2	30.3	30.3
7	18.9	14.9	47.8	51.8	19	79.3	61.9	10.1	27.5
8	19.0	14.5	44.4	48.9	20	14.0	12.6	32.6	34.0
9	62.0	51.8	22.0	32.2	21	75.1	63.7	11.5	22.9
10	55.6	42.6	20.5	33.5	22	83.9	62.7	8.0	29.2
11	65.0	58.5	20.8	27.3	23	26.2	19.5	47.0	53.7
12	73.6	60.9	15.7	28.4	24	81.1	63.4	8.3	26.0

In this table the most pronounced variation occurs in sample 22. Obviously our definition of soil texture can become very misleading if we describe it as containing 8.0 per cent fine sand, whereas it should rightly contain 29.2 per cent.

It is suggested that the particles of fine sand whose nodal fractions are approximately 0.2 mm. in diameter are held too securely by surface tension to be removed by wet sieving. In order to be removed they must be allowed freedom of movement, and for this reason much the same result as dry sieving can be obtained by sieving under water.

However this may be, it is evident that if the widely accepted definition of fine sand is meant to imply that fraction which passes through a 0.2 mm. sieve, then the usual technique does not produce results which always comply with that definition. The procedure of dry sieving which ensures a correct separation does not involve further work, and has been the routine of this laboratory for some time past.

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# DIGESTIBILITY TRIALS WITH POULTRY

## VII. THE DIGESTIBILITY OF WHEAT OFFALS, WITH A NOTE ON THE APPARENT DISCREPANCY BETWEEN THE DIGESTI- BILITY COEFFICIENTS AND NUTRITIVE VALUES OF THESE PRODUCTS

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### INTRODUCTION

WHEAT intended for the manufacture of flour is subjected by the millers to a preliminary process of cleaning. This cleaning process includes screening, washing and conditioning, the screening and washing processes serving to remove the impurities present in the bulk samples. In conditioning, the moisture content of the grain is adjusted to the point which practice has found to be necessary to ensure successful milling of the sample in question. The cleaned and conditioned grain is then subjected to the milling process proper, which consists of alternate grinding and separation processes which are repeated until the object of the miller is achieved, i.e. the production of the maximum amount of high-grade flour with the minimum contamination of "wheat offals". In modern milling the grinding processes are carried out by means of steel rolls, two kinds of which are used, ribbed or fluted rolls known as "break" rolls, and smooth rolls known as "reduction" rolls. The speed and distance apart of each pair of rolls are so adjusted as to effect the break up of the grain with the production of minimum compression or friction, since compression or friction are fatal to the production of a high-class flour. For the separation processes silk bolting cloths and sieves and forced air draft are used. The character and nature of the by-products produced vary considerably from mill to mill according to the nature of the processes adopted by the millers and according to the extent to which intermixing of the grades of by-products produced takes place. Owing to the indefinite grading of these wheat products and the use of local names, the task of assigning a definite value to them is almost impossible except in the case of bran. Wood & Adie (1917), in a careful and comprehensive investigation of the subject, showed that the offals sold in England and Wales could be classified into three pure grades of the finer

offals and three "mixed" grades which consist of admixtures of the three pure grades. The three pure grades consist of pollards (local names—randans, coarse sharps and gurgeons), coarse middlings (local names—sharps, thirds, boxings and parings) and fine middlings (local names—seconds, fine thirds and biscuit middlings). The mixed grades consist of straight-run middlings (fine middlings and coarse middlings), straight-run pollards (coarse middlings and pollards) and straight-run offals (pollards, coarse and fine middlings). Owing to the extent to which wheat offals are used in poultry mashes it was considered desirable to complete the information already obtained on the digestibility of various wheat varieties by ascertaining the digestibility of some of these by-products.

The wheat by-products used in the experiment were purchased locally and were sold as broad bran, coarse middlings and fine middlings respectively. These products gave the following analysis.

	Broad bran	Straight-run pollards (coarse middlings)	Fine middlings
Moisture	13.34	12.91	13.27
Protein	14.38	16.44	8.38
Ether extract	3.66	4.35	2.26
N-free extract	52.52	56.42	72.34
Fibre	10.30	6.14	1.92
Ash	5.80	3.74	1.83
	100.00	100.00	100.00

The analyses, when compared with the representative analyses given by Wood & Adie, revealed the fact that the coarse middlings purchased was not in reality a pure-grade middlings at all, and should be classified as straight-run pollards and not as coarse middlings. For this reason the so-called coarse middlings is described throughout under its technically true name, straight-run pollards.

Four Light Sussex cockerels were used as experimental animals, and the collection period was extended over 16 days in every case. Care was taken throughout the trials to ensure that equal quantities of food were consumed at each meal, and a preliminary feeding period of 7 days elapsed before the commencement of collection of the food residues. This preliminary feeding period is of considerable importance, since observation has shown that, in change-over from one food to another, a retention of fibre often occurs in the gizzard, necessitating a comparatively long preliminary feeding period in order to ensure that the food residues are truly representative of the particular food under trial.

The birds were fed twice daily, and the material in each case was moistened before feeding in order to avoid possible mechanical loss. In

the case of the bran period it was found impossible to ensure a consumption of more than 60 g. per day, but in the case of the straight-run pollards and the fine middlings 100 g. per day were given. The birds kept in healthy condition throughout the trials, and gained weight during both the straight-run pollards period and the fine middlings period. In the bran period, however, the amount of food consumed was evidently insufficient for maintenance, since the birds lost weight while under experiment.

The experimental and analytical procedure adopted were the same as outlined in a previous communication (Halnan, 1926), except that, through the kindness of the late Sir William Hardy, facilities were provided by the Low Temperature Research Station for the storage of the freshly collected excreta at low temperature.

#### EXPERIMENTAL DATA OF DIGESTIBILITY TRIALS

##### (1) *The digestibility of broad bran*

Period of experiment: 16 days. Food fed: 960 g. per bird

Average composition of mixed excreta in g. actual weight

	Dry excreta	Organic matter	Total nitrogen	Uric acid nitrogen	Ammoniacal nitrogen	Ether extract	Crude fibre	Ash
Bird 1	648.4	579.8	45.29	27.71	4.55	17.26	90.42	68.6
Bird 2	627.0	560.1	41.65	25.26	4.32	18.28	87.71	66.9
Bird 3	624.2	551.9	28.62	14.11	2.94	17.73	89.82	72.3
Bird 4	613.5	545.9	28.67	14.74	2.46	18.71	91.28	67.6

##### *Digestibility coefficients of broad bran*

###### Bird 1

Weights in g.

	Organic matter	Nitrogen in crude protein	Ether extract	Crude fibre	Nitrogen-free extract
Food contains	776.3	22.08	35.13	98.88	504.2
Dung contains	459.9	8.51	15.10	90.42	301.2
Digested	316.4	13.57	20.03	8.46	203.0
Digestibility coefficient	40.8%	61.50%	57.00%	8.50%	40.5%

###### Bird 2

Food contains	776.3	22.08	35.13	98.88	504.2
Dung contains	450.2	7.93	16.30	87.71	296.6
Digested	326.1	14.15	18.83	11.17	207.6
Digestibility coefficient	42.0%	64.10%	53.60%	11.30%	41.2%

###### Bird 3

Food contains	776.3	22.08	35.13	98.88	504.2
Dung contains	488.9	9.29	16.60	89.82	324.4
Digested	287.4	12.79	18.53	9.06	179.8
Digestibility coefficient	37.0%	57.90%	52.70%	9.20%	35.7%

## Bird 4

## Weights in g.

	Organic matter	Nitrogen in crude protein	Ether extract	Crude fibre	Nitrogen- free extract
Food contains	776.3	22.08	35.13	98.88	504.2
Dung contains	482.3	9.17	17.57	91.28	316.1
Digested	294.0	12.91	17.56	7.60	188.1
Digestibility coefficient	37.9 %	58.50 %	50.00 %	7.70 %	37.4 %
Average digestibility coefficient	39.4 %	60.50 %	53.30 %	9.20 %	38.7 %

(2) *The digestibility of straight-run pollards*

Period of experiment: 16 days. Food fed: 1600 g. per bird

Average composition of mixed excreta in g. actual weight

	Dry excreta	Organic matter	Total nitrogen	Uric acid nitrogen	Ammoniacal nitrogen	Ether extract	Crude fibre	Ash
Bird 1	652.9	580.2	36.51	21.25	2.90	15.37	89.35	72.7
Bird 2	637.7	577.7	36.80	21.39	3.45	16.84	92.86	60.0
Bird 3	679.5	601.3	37.88	22.13	3.83	15.49	94.71	78.2
Bird 4	654.7	569.9	35.41	21.13	3.22	15.99	94.43	84.8

*Digestibility coefficients of straight-run pollards*

## Bird 1

## Weights in g.

	Organic matter	Nitrogen in crude protein	Ether extract	Crude fibre	Nitrogen- free extract
Food contains	1333.6	42.08	69.60	98.24	902.7
Dung contains	490.6	9.03	13.76	89.35	331.0
Digested	843.0	33.05	55.84	8.89	571.7
Digestibility coefficient	63.2 %	78.50 %	80.20 %	9.10 %	63.3 %

## Bird 2

Food contains	1333.6	42.08	69.60	98.24	902.7
Dung contains	485.5	8.52	15.19	92.86	324.1
Digested	848.1	33.56	54.41	5.38	578.6
Digestibility coefficient	63.5 %	79.80 %	78.20 %	5.50 %	64.1 %

## Bird 3

Food contains	1333.6	42.08	69.60	98.24	902.7
Dung contains	504.9	8.32	13.75	94.71	344.4
Digested	828.7	33.76	55.85	3.53	558.3
Digestibility coefficient	62.1 %	80.20 %	80.20 %	3.60 %	61.9 %

## Bird 4

Food contains	1333.6	42.08	69.60	98.24	902.7
Dung contains	479.5	7.68	14.36	94.43	322.7
Digested	854.1	34.40	55.24	3.81	580.0
Digestibility coefficient	64.0 %	81.70 %	79.40 %	3.90 %	64.3 %
Average digestibility coefficient	63.2 %	80.00 %	79.50 %	5.40 %	63.4 %

*Digestibility Trials with Poultry**(3) The digestibility of fine middlings*

Period of experiment: 16 days. Food fed: 1600 g. per bird

Average composition of mixed excreta in g. actual weight

	Dry excreta	Organic matter	Total nitrogen	Uric acid nitrogen	Ammoniacal nitrogen	Ether extract	Crude fibre	Ash
Bird 1	301.8	273.2	30.37	18.40	1.46	4.26	30.07	28.6
Bird 2	307.9	281.6	33.71	20.78	3.53	3.63	27.69	26.3
Bird 3	344.8	314.2	33.01	20.61	3.47	4.37	29.73	30.6
Bird 4	337.3	308.2	31.64	18.95	3.38	4.18	31.21	29.1

*Digestibility coefficients of fine middlings*

## Bird 1

Weights in g.

	Organic matter	Nitrogen in crude protein	Ether extract	Crude fibre	Nitrogen-free extract
Food contains	1358.4	21.44	36.16	30.92	1157.4
Dung contains	196.0	6.69	2.87	30.07	121.2
Digested	1162.4	14.75	33.29	0.65	1036.2
Digestibility coefficient	85.5%	68.80%	92.10%	2.10%	89.5%

## Bird 2

Food contains	1358.4	21.44	36.16	30.92	1157.4
Dung contains	191.2	5.99	2.00	27.69	124.1
Digested	1167.2	15.45	34.16	3.03	1033.3
Digestibility coefficient	85.9%	72.10%	94.50%	9.90%	89.3%

## Bird 3

Food contains	1358.4	21.44	36.16	30.72	1157.4
Dung contains	224.6	5.53	2.76	29.73	157.6
Digested	1133.8	15.91	33.40	0.99	999.8
Digestibility coefficient	83.5%	74.20%	92.40%	3.20%	86.4%

## Bird 4

Food contains	1358.4	21.44	36.16	30.72	1157.4
Dung contains	225.2	6.19	2.69	31.21	152.6
Digested	1133.2	15.25	33.47	—	1004.8
Digestibility coefficient	83.4%	71.10%	92.60%	—	86.8%
Average digestibility coefficient	84.6%	71.50%	92.90%	3.40%	88.0%

## DISCUSSION OF RESULTS

Owing to the variability of composition of wheat milling by-products other than bran, it was considered useless to attempt to correlate the results of previous experiments with the results here reported except in the case of bran itself, which is a product of fairly constant composition. The first point of interest that arises is the relative ineffectiveness of the bird's powers of digestion of bran compared with other livestock. In a previous communication (Halnan, 1928) it was pointed out that, in the case of products low in fibre, the powers of digestibility possessed by

poultry were akin to those of the pig. It has further been demonstrated that the presence of fibre in poultry feeding stuffs leads to a depression of digestibility. It would therefore be expected that, as the fibre increases in a feeding stuff, a divergence would be shown between the digestibility of such feeding stuffs by poultry and that by other animals. Comparison of the figures given in the table which follows shows this clearly to be the case.

*Percentage digestibility coefficients of bran by various classes of livestock*

	Organic matter	Crude protein	Crude fibre	Ether extract	Nitrogen-free extract
Poultry (Halnan)	39.4	60.5	9.2	53.3	38.7
Poultry (Fraps, 1928)	—	59.9	7.9	50.0	54.1
Pigs (Kellner)	67.0	75.0	33.0	72.0	66.0
Pigs (Honcamp, 1913)	63.0	75.7	—	72.4	64.1
Sheep (Köhler, 1903)	65.9	72.6	32.5	81.5	68.8
Sheep (Honcamp, 1913)	72.4	77.2	54.4	80.7	74.1

These figures also clearly bring out the fact that bran is digested to a much lesser extent by poultry than by other livestock, and its use for poultry as a source of energy would appear to be economically unsound as long as a demand for its use by other livestock exists. Comparison with the digestibility coefficients of the finer wheat offals brings out this point still more clearly.

*Digestibility coefficients of wheat offals by poultry*

	Organic matter	Crude protein	Crude fibre	Ether extract	Nitrogen-free extract
Broad bran	39.4	60.5	9.2	53.3	38.7
Straight-run pollards	63.2	80.0	5.4	79.5	63.4
Fine middlings	84.6	71.5	3.4	92.9	88.0

The organic matter, the crude protein, the ether extract and the nitrogen-free extract are much more efficiently digested in the case of the finer wheat offals, and from these results it would appear that the use of these finer grades of wheat offals as a source of energy for poultry is preferable to bran. Calculated on a moisture-free basis, 100 lb. of bran yield 38 lb. of starch equivalent, 100 lb. of straight-run pollards 64.4 lb. and 100 lb. of fine middlings 85.4 lb. From the point, therefore, of the fattening capacity of these foods, the finer wheat offals should be used in preference to the bran. Moreover, comparison of these figures with those representing the utilization of these products by other farm animals shows that, whereas bran is inferior, the finer grades of wheat offals are utilized quite as efficiently by poultry as by other farm animals; they therefore form an extremely useful source of food supply for poultry.

A NOTE ON THE APPARENT DISCREPANCY BETWEEN THE VALUE OF WHEAT  
OFFALS AS ASSESSED BY DIGESTIBILITY DETERMINATIONS  
AND THEIR VALUE IN ACTUAL PRACTICE

In view of the definite opinion held by practical poultry keepers that bran is a valuable feeding material for poultry, it was decided that this point should be further explored before publishing the results obtained by the digestibility trials. Two points appeared to be at issue: one, the value of bran as a fattening material, and two, its value from a dietetic standpoint. At the outset it may be as well to emphasize the fact that a digestibility determination does not give a measure of all the factors upon which the nutritive value of a food depends. In actual fact it gives us a measure of the amount of digestible protein and energy-producing substances present in 100 lb. of the feeding stuff. It gives no information on the vitamin content of the food, the nature and availability of the mineral substances present, the quality of the protein present, nor on the palatability of the food itself. All these latter factors are of extreme importance in assessing the nutritive value of a food, and, under certain conditions, may determine whether the animal can utilize efficiently the digestible nutrients made available to it. In connexion with a proposed investigation into the nutritive value of wheat and its by-products, a preliminary series of trials was undertaken to ascertain whether mixtures of wheat and its by-products could be used for raising chicks under battery brooder conditions. The birds were divided into two groups and were reared throughout in a Gloucester electrically heated brooder. In the first experiment both groups received rations consisting of cut wheat + 1.5 per cent cod-liver oil + flint grit + 2 per cent oyster shell. Group 1 was given cut green food (kale) in addition. At 4 weeks of age 10 per cent of dried milk powder was added to the rations. The foods were dry fed *ad libitum*. In Group 1 of 48 original chicks 26 survived to the end of the experiment. In Group 2 of 47 original chicks 27 survived to the end of the experiment. The average weights of the survivors were as follows:

	At beginning (5 days old)	At time of change to 10 % milk (28 days old)	At end of trial (61 days old)
	g.	g.	g.
Group 1	41	73	341
Group 2	41	66	335

Owing to the small number of chicks used it is not proposed to attach much importance to the actual differences of weights obtained in the two groups. The results, however, do indicate that wheat supplemented

with cod-liver oil and calcium carbonate is deficient in factors required for the normal growth of chicks, that these factors are to a large extent present in dried milk and that green food does not supply the factors that are lacking. It should be added that the chicks readily ate the cut wheat, that feathering was poor in both groups, but the chicks in Group 1 feathered more satisfactorily than Group 2.

Experiment 2 was designed to compare the growth effects of weatings and cut wheat. In this case the cut wheat was roughly ground into a meal. At 3 days old 86 chicks were divided into two groups. Group 1 received a mixture of weatings + 2 per cent oyster shell + 1.5 per cent cod-liver oil, and Group 2 cut wheat + 2 per cent oyster shell + 1.5 per cent cod-liver oil. The foods were dry fed. Within 2 days from the commencement of the experiment trouble was experienced in the case of the weatings group, due to pasting up of the weatings on the beaks. This pasting resulted in the formation of hard pads of food material on the inside of the upper and lower beaks, and accumulated so rapidly as to prevent the chicks closing their mouths. Frequent cleaning of the mouths had no effect, and 7 days after the commencement of the experiment sores had developed in the angles of the jaw in many cases. On this day the weatings was changed to 50 per cent weatings and 50 per cent bran in the hope that the trouble might be overcome. Although the trouble with beak clogging was diminished during the latter part of the experiment, a few cases still occurred. It is noteworthy that only one case of beak clogging occurred in the ground-wheat group. In the case of the ground-wheat group 10 per cent of milk powder was added to the ration on the 24th day (3 weeks after the commencement of the experiment). The same effect on the growth rate was noted as in Experiment 1, the chicks which up to this time had been growing at a very slow rate at once improved. On the 38th day of the experiment cannibalism began to make its appearance in this group, seven birds in all becoming incapacitated through this cause. In Group 1 10 birds survived out of 43, and in Group 2 24 survived out of 43. The average weights of the survivors were as follows:

	At beginning (3 days old)	10 % milk added to Group 2 (24 days old)	At end of trial (52 days old)
	g.	g.	g.
Group 1	39	114	369
Group 2	37	59	216

Owing to the trouble with the beak clogging in the weatings group with the resultant large percentage of deaths, it is not possible to attach



much importance to the average weights given, but it is significant that the survivors, who were not troubled with beak clogging, made good growth. Close observation of the chicks during feeding revealed the fact that the relatively small number of chicks which showed no signs of beak clogging did not feed from the trough in the earlier stages of the experiment, but took their food from the beaks of their more unfortunate companions. Their immunity from the trouble was undoubtedly due to this cause.

The results of this experiment conclusively proved that weatings supplemented with oyster shell and cod-liver oil is unsuitable by itself as a chick feed owing to its inherent property of pasting into a sticky, doughy mass when wetted, and it is possible that the cases of clogged beaks that have occurred in battery brooded chicks reared under commercial conditions may in certain instances have been due to including too large a proportion of this ingredient in the dry mash. In the third experiment it was decided to test the growth effect of bran against a bran-weatings mixture (60 per cent bran and 40 per cent weatings). 55-day-old chicks of various breeds were fed for 8 days on bran supplemented with 1.5 per cent cod-liver oil. The 49 survivors at the end of this period were divided into two groups, Group 1 receiving bran + 1.5 per cent cod-liver oil + 2 per cent oyster shell, Group 2 receiving bran 60 per cent + weatings 40 per cent + 1.5 per cent cod-liver oil + 2 per cent oyster shell. The bran and weatings used were freshly milled and supplied through the courtesy of the Millers Mutual Association. The analyses were as follows:

	Bran	Weatings	60 % bran + 40 % weatings
Moisture	9.95	10.55	10.19
Ether extract	3.40	4.10	3.68
Crude protein	14.70	15.14	14.88
N-free extract	60.27	62.27	61.07
Fibre	7.05	4.98	6.22
Ash	4.63	2.96	3.96

During the experiment only one case occurred of beak clogging, the chicks maintained good appetites throughout, and normal gains resulted. In addition, the birds feathered extremely well, and the death-rate was lower than in the previous experiments: Group 1, 19 survivors out of 24; Group 2, 16 survivors out of 25. The average weights of the survivors were as follows:

Age in days ...	9	16	23	30	37	44	51	58
Group 1 (in g.)	47	68	99	144	194	267	335	366
Group 2 (in g.)	45	64	86	115	157	210	274	349

It will be noted that the bran group during the earlier stages made better gains than the bran and weatings group. The growth-rate of the bran and weatings group was, however, of a more even character than the bran group, and towards the end of the experiment these chicks were making better gains than the bran group. It was further noted that the bran-fed chicks in the latter stages of the experiment were almost continually on feed.

Summarizing the results obtained in the chick feeding experiments, it would appear that, from a growth standpoint, wheat by-products such as bran and weatings are better than wheat itself. The inclusion of weatings in a chick mixture to an extent exceeding 40 per cent would appear to be inadvisable owing to its inherent tendency to clog the beak, a tendency that is absent both in the case of bran or coarsely ground wheat. Bran would also appear to be as good as, if not better than weatings as a promoter of growth. Since, in the digestibility trials dealt with earlier in this paper, on a moisture-free basis 100 lb. of bran yield 38 lb. of starch equivalent, whereas straight-run pollards yield 64.4 lb. of starch equivalent, the growth results would appear to be at variance with the digestibility results. In actual fact, however, this is not so. The chicks in the bran group consumed 54,625 g. of bran, whereas the chicks in the bran and weatings group consumed 24,375 g. The total gains in live-weight increase (including those birds which died during the experiment) were 5273 g. in the bran group and 4898 g. in the bran and weatings group. The amounts of bran and bran-weatings mixture consumed per lb. live-weight increase accordingly work out at 10.35 and 4.97 lb. respectively. These results fall into line with the relative starch-equivalent values for bran and middlings obtained in the digestibility determinations; and the apparent equivalent nutritive values of bran and middlings is really due, not, as appears at first sight, to the fact that equal amounts of bran and middlings have equivalent values, but owing to the fact that the birds consume in the same period of time at least twice the amount of bran as they do the bran-middlings mixture. Indeed, the results obtained indicate that a mixture of bran and middlings forms a biologically better balanced food than either of these foods fed separately.

## SUMMARY OF CONCLUSIONS

1. Digestibility determinations of broad bran, straight-run middlings and fine middlings are here reported.
2. On the basis of these determinations bran would appear to be a relatively dear feeding stuff for use with poultry, as compared with the finer grades of milling offals.
3. In the case of chick feeding mixtures, the finer milling offals suffer from the disadvantage of causing pasting in the mouth, this disadvantage militating against their too extensive inclusion in chick feeding mixtures. From the evidence obtained in these experiments it would appear that this disadvantage would come into play when more than 40 per cent of the total mixture consists of the finer offals.
4. From the point of view of economy and efficiency of utilization, a mixture of bran and middlings would appear to be of more value than either of these foods fed separately.
5. The inclusion of bran in chick feeding mixtures would appear to be justified on dietetic grounds, in spite of its relatively poor value measured by digestibility trials.
6. From a biological standpoint, bran and bran-weatings mixtures appear to be definitely superior to wheat itself when used exclusively as a source of food and supplemented with 1.5 per cent cod-liver oil and 2 per cent oyster shell.

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# DIGESTIBILITY TRIALS WITH POULTRY

## VIII. THE DIGESTIBILITY OF DRIED MOLASSED SUGAR-BEET PULP

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### INTRODUCTION

IN the process of manufacture of sugar from sugar beet, the sugar is first extracted from the beet by appropriate means, and the wet residue or pulp is then dried and sold for cattle feeding. The composition of the dried pulp or slices differs somewhat according to the nature of the process used in the extraction of the sugar. In the "diffusion" process, in which water is used, the extraction is very efficient, with the result that the sugar content of the dried pulp is low. In the Steffen process, in which press methods are used, a fair amount of sugar is left in the dried residues. In the de Vecchi process the sliced beets are dried before the extraction of the sugar takes place, and a certain proportion of these slices is occasionally marketed on the Continent as cattle food. The characteristic differences between these three types of pulp are shown by analyses given by Fangauf and Waldow (1).

### *Analyses of dried sugar-beet pulp*

	Moisture	Protein	Fat	Carbo- hydrates	Fibre	Ash
Sugar extracted pulp:						
Diffusion process	8.26	10.22	0.50	59.96	14.98	6.08
Steffen process	10.17	6.19	0.41	66.27	12.17	4.79
Unextracted pulp	4.36	5.73	0.32	83.07	4.35	2.17

In this country the dried pulp which finds its way to the market is generally produced by the diffusion process, and is either marketed as produced, or, as will be seen later, after the incorporation into it of the molasses arising during the process of refining the beet sugar.

A certain body of evidence has been accumulated in this country on the value of dried sugar-beet pulp for stock feeding. Johnston(2), from the result of 3 years' bullock-feeding experiments, came to the conclusion that 1 ton of dried pulp was equivalent in feeding value to approximately 7 tons of swedes. The condition of the carcasses of the pulp-fed bullocks

was in no way inferior to that of bullocks fed on other foods. Gardner & Hunter-Smith<sup>(3)</sup> found that, for the fattening of cattle of the "baby beef" class, crushed oats, dried sugar-beet pulp, and molassed sugar-beet pulp might be regarded as of equal feeding value. Woodman, Mansfield & Garner<sup>(4)</sup>, in a fattening trial with bullocks of the Shorthorn and Lincoln Red type, found that molassed beet pulp and dried sugar-beet pulp were of equal feeding value to oats. The carcasses of the beet-fed bullocks killed well and were of very satisfactory quality. Up to 15 lb. of dried pulp per head per day was fed in the finishing rations with highly satisfactory results. Woodman & Calton<sup>(5)</sup>, from digestibility trials with sheep, ascertained that sugar-beet pulp is highly digestible by ruminants, and suggested that dried sugar-beet pulp should be regarded as a carbohydrate concentrate, 1 lb. of which was equivalent to 0.8 lb. of maize or 0.9 lb. of barley. Tilley<sup>(6)</sup>, from the evidence presented by the results of 2 years' feeding trials with dairy cows, came to the conclusion that 1 ton of molassed beet pulp was equivalent in feeding value to 7 tons of mangels or 1 ton of crushed oats, and found that the feeding of the molassed beet pulp did not give rise to taint in the milk. It is clear, from the above, that dried sugar-beet pulp and molassed sugar-beet pulp are of equivalent feeding value in ruminants, and that they are both equal in feeding value to crushed oats.

The evidence with regard to the value of these products in the nutrition of swine is not so favourable. Woodman, Duckham & French<sup>(7)</sup> found that, although pigs were only to a slight extent inferior to ruminants in their powers of digesting sugar-beet pulp and molassed sugar-beet pulp, the results obtained in feeding trials with pigs were decidedly inferior to those obtained with ruminants. The authors associated this inferiority with the bulky character, when soaked, of the feeding stuffs in question, thus resulting in diminished intake of food with a subsequent diminution of live-weight increase, and suggested that these materials may be of use, when given in moderate quantity, as a food for breeding stock or for pigs in which maximum increase of live weight is not the object aimed at.

The fact that sugar-beet pulp has been proved to be a satisfactory feeding stuff for use in the rations of bullocks, dairy cows and sheep, but of inferior value in the case of swine, which possess a relatively simpler digestive apparatus, led the author to undertake the present investigation with fowls, in which the structure of the digestive tract is simpler than that of swine, and through which the passage of food is relatively rapid.

Through the courtesy of Sugar Beet Products, Ltd., a bag of molassed

sugar-beet pulp was supplied for the purpose of the trial, and the author wishes to express his acknowledgements for the gift of this material and for information regarding its manufacture.

#### PROCESS OF MANUFACTURE

The wet slices or residue of the sugar beet remaining after the extraction of the sugar are passed through conveyers through which the molasses are allowed to enter. By means of a worm screw, the pulp and the molasses are thoroughly mixed together, and the mixture is then fed into a cylindrical drum drier, where it is exposed to the action of hot air. During the process of drying, the wet pulp and molasses are in a state of constant movement, thus bringing every portion of the mixture into intimate contact with the hot air which quickly dries it. As the dried molassed pulp moves through the cylinder, the temperature is gradually reduced, so that when it arrives at the discharge point it is ready to be bagged.

#### DESCRIPTION OF TRIALS

For the purpose of the trials four Light Sussex cockerels were used. Each cockerel was housed in a roomy wire cage fitted underneath with a plate-glass slide so as to facilitate quantitative collection of the excreta. Since the birds would not eat the dried pulp when fed by itself, fine middlings, the digestibility of which had been previously determined, were mixed with it. Moreover, since pulp swelled considerably when wetted, the consumption of pulp had necessarily to be restricted to 10 g. of the dried meal daily. The sample of dried pulp was first ground to a meal, and in the case of each bird 10 g. of the meal were mixed with 40 c.c. of water and allowed to soak overnight. The following morning 40 g. of fine middlings were mixed in and the resultant wet mash fed to the bird in two portions. After a preliminary feeding period of 4 days, collection of the excreta began. The same procedure with regard to collection and analysis of the excreta was followed as in previous trials. The experimental period proper lasted 16 days.

#### EXPERIMENTAL DATA

##### *Analysis of dried molassed pulp*

Moisture	...	...	6.25
Crude protein	...	...	9.88
Ether extract	...	...	0.63
Carbohydrates	...	...	64.27
Fibre	...	...	13.58
Ash	...	...	5.39
100.00			

*Digestibility Trials with Poultry*

Period of experiment: 16 days. Food fed: molassed pulp, 160 g.; fine middlings, 640 g.

Average composition of the mixed excreta in g.

	Dry excreta	Organic matter	Total nitrogen	Uric acid nitrogen	Ammoniacal nitrogen	Ether extract	Crude fibre	Ash
Bird 1	281.44	255.21	22.23	13.88	1.96	3.24	36.04	26.23
Bird 2	288.20	264.40	22.07	12.10	2.85	2.84	35.90	25.80
Bird 3	300.21	270.86	23.42	14.72	2.43	3.42	38.79	29.35
Bird 4	266.70	243.10	19.21	11.64	1.00	3.10	36.15	23.60

*Digestibility coefficients of dried molassed pulp*

## Bird 1

Weights in g.

	Organic matter	Nitrogen in crude protein	Ether extract	Crude fibre	Nitrogen- free extract
Food contains	684.80	11.11	15.47	34.03	568.10
Dung contains	196.30	4.17	2.18	36.04	132.10
Digested	488.50	6.94	13.29	—	436.00
Due to middlings	464.70	5.90	13.31	—	416.60
Due to pulp	23.80	1.04	—	—	19.40
Digestibility coefficient	16.83 %	41.10 %	—	—	18.86 %

## Bird 2

Food contains	684.80	11.11	15.47	34.03	568.10
Dung contains	206.60	4.95	1.84	35.90	137.90
Digested	478.20	6.16	13.63	—	430.20
Due to middlings	466.90	6.18	13.66	—	415.50
Due to pulp	11.30	—	—	—	14.70
Digestibility coefficient	8.00 %	—	—	—	14.29 %

## Bird 3

Food contains	684.80	11.11	15.47	34.03	568.10
Dung contains	207.10	3.86	2.27	38.89	141.90
Digested	477.70	7.25	13.20	—	426.20
Due to middlings	453.50	6.37	13.36	—	402.00
Due to pulp	24.20	0.88	—	—	24.20
Digestibility coefficient	17.12 %	34.78 %	—	—	23.53 %

## Bird 4

Food contains	684.80	11.11	15.47	34.03	568.10
Dung contains	192.00	3.54	2.18	36.15	131.50
Digested	492.80	7.57	13.29	—	436.60
Due to middlings	453.30	6.10	13.38	—	403.90
Due to pulp	39.50	1.47	—	—	32.70
Digestibility coefficient	28.00 %	58.10 %	—	—	31.80 %
Average digestibility coefficient	17.50 %	33.50 %	Nil	Nil	22.12 %

## DISCUSSION OF RESULTS

From the average digestibility coefficients obtained it would appear that the digestibility of this material by fowls is extremely poor. This is particularly the case with the nitrogen-free extract, a rather remarkable result in view of the fact that a proportion of this nutrient consists of sugar with a presumably high digestibility coefficient. If, however, the conditions under which the digestibility trial had to be undertaken are considered, a possible explanation of the results obtained presents itself. As mentioned earlier, it was found impossible to feed the molassed beet pulp alone, and for this reason a mixture of 75 per cent middlings and 25 per cent beet pulp was fed. The digestibility of the middlings had been previously obtained; in that case the middlings had formed the sole source of the diet. The digestibility figures for the middlings so obtained had necessarily to be taken when estimating the digestibility of the beet pulp by difference, and it follows that any depression in the digestibility of the middlings caused by its admixture with the beet pulp would cause the apparent digestibility of the beet pulp to appear lower than it actually is. Previous work had already shown that the digestibility of a food by fowls is correlated with the fibre content of the food, the higher the fibre the lower the digestibility of the other ingredients. The conditions under which the middlings were fed in this experiment were akin to feeding the middlings with added fibre. The strong probability exists, therefore, that the actual digestibility of the nutrients of the middlings when fed with the sugar-beet pulp was less than it was when the middlings were fed *per se*, but since the digestibility of the middlings had to be taken on the assumption that no such depression had taken place, the net effect would be to make the beet pulp appear to be much less digestible than it actually is.

Since, however, it is difficult to conceive a situation in which sugar-beet pulp is used as a sole ingredient of a poultry ration, it becomes legitimate to charge the sugar-beet pulp itself with the depression of digestibility it apparently causes when admixed with other foods. For this reason it is considered justifiable to accept the digestibility figures obtained in this experiment.

Fangauf & Waldow<sup>(1)</sup> studied the effect on egg production of (a) slices made by the Steffen process, (b) full value slices made from cleaned beets cut and dried, and (c) slices made from the leached residue after sugar extraction. The birds were Leghorns, and the dried slices were mixed with a mash consisting of wheat meal 15 per cent, rice meal



15 per cent, barley meal 10 per cent, maize meal 10 per cent and fish meal 25 per cent, in the proportion of 25 per cent of dried slices to 75 per cent of the mash. 50 g. of grain per head were fed in addition to the mash, which itself was fed in a damped condition. The main facts which were obtained are outlined in the table which follows:

	Slices by Steffen process	Full value slices	Extracted slices
Average consumption per head per day of slices (g.)	12.7	12.0	11.2
Average egg production (Jan.-July inclusive)	138.4	127.3	118.9
Average percentage hatchability based on:			
(a) eggs laid	80	75.8	62.4
(b) eggs fertile	92.6	79.7	65.3

The results of this experiment showed that sugar-beet slices containing a certain proportion of sugar can be successfully included in a mash ration without any apparent marked detriment to either egg production or hatchability. On the other hand, dried sugar-beet slices made from the leached residue from sugar extraction proved to be an unsuitable food material, since the authors noted that the use of this material caused an unpleasant smell in the poultry shed, a fall in body weight of the birds accompanied with an unthrifty appearance, and reduced hatchability of the eggs produced. It would consequently appear, both from the digestibility and the dietetic standpoints, that sugar-beet slices are unsuitable for poultry feeding, except in times of scarcity of carbohydrate containing foods. Moreover, in view of the efficiency of ruminants in dealing with this class of feeding stuff, it would appear to be economically unsound to use it for any other class of stock than ruminants.

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# ARTIFICIAL INSEMINATION OF SHEEP

## I. PRELIMINARY INVESTIGATION ON ITS APPLICATION TO SHEEP BREEDING IN KENYA

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IN recent years artificial insemination has been very successfully applied to livestock breeding in Russia. In 1932, 920,000 ewes were inseminated with a conception rate of 82·4 per cent, which compares very favourably with the percentage of conceptions (79·6) in 1,560,000 ewes served normally (Ozin & Parsutin, 1934). In 1933 the percentage of conceptions in 1,500,000 sheep inseminated was equal to, or above, that of normal service (Parsutin, 1934). The percentage of fertilization after artificial insemination in 31,911 Karakul ewes was 79·42 (Kuznetzova, 1932). On the other hand Demidenko *et al.* (1933) with a flock of 3459 ewes obtained 57·8 per cent of conceptions following artificial insemination as opposed to 79·0 after normal service. It is probable that the successful application of artificial insemination to livestock breeding in the Colonies and other overseas countries would be of considerable value, and the experiment which is described in this paper was designed to explore these possibilities as far as sheep breeding in Kenya is concerned.

### EXPERIMENTAL MATERIAL AND METHODS

The technique adopted was essentially that of Russian workers described in the monograph of the Imperial Bureau of Animal Genetics (1933). The collection of sperm was easily effected by means of an artificial vagina (Walton's pattern). Prior to use, the inner surface of the rubber lining was swabbed with 65 per cent alcohol, allowed to dry and smeared with white vaseline. The space between the cylinder and the inner tube was filled with warm water at a temperature of about 40–50° C. The correct distension of the tube was then obtained by blowing air into the space between the cylinder and the tube. The artificial vagina on account of its small size cools down fairly quickly and it is therefore considered advisable to warm the cylinder with hot water before finally filling it with water at a temperature of 40–50° C.

In the large majority of cases the ram from which it was desired to

collect sperm was placed with a ewe on heat, which had been picked out by a "teaser" ram. It does not, however, seem to be essential that a ewe used for this purpose should be on heat, since on several occasions a ewe which was not on heat has been used successfully. If the ewe is held to prevent her moving, a ram accustomed to the procedure will mount her.

Sperm was microscopically examined immediately after collection for motility and density. It was then diluted and examined microscopically as a check on the effect of the dilutor on the sperm; a further microscopic examination was made on any sperm remaining after the insemination had been carried out. No specimens of sperm from the ram chosen for the insemination had to be discarded because of poor quality. The maximum time that elapsed between collection of sperm and the end of the insemination was an hour, but in the majority of cases the insemination was usually completed in about half an hour.

The dilutor used was GPS-2 (Milovanov & Selivanova, 1932), which has the following composition:

*Solution I* (g. per litre of water)

Anhydrous glucose	64.0
$\text{KH}_2\text{PO}_4$	1.7

*Solution II* (g. per litre of water)

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	41.6
$\text{CaHPO}_4$	0.1
$\text{MgHPO}_4$	0.1

These solutions were made up with distilled water that had been boiled to expel  $\text{CO}_2$ . The equivalent amount of anhydrous disodium hydrogen phosphate (B.D.H.) was used in place of the  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ . The pH of the dilutor was tested with a phenol red capillator. This should be 7.6. The dilutor was kept in test-tubes or flasks plugged with cotton wool. Sterilization was carried out immediately after preparation in a steam sterilizer; this was done for 20 min. on three successive days. For use, equal amounts of solutions I and II were taken and mixed, sufficient dilutor being prepared according to the dilution required. Flasks were sterilized daily after withdrawal of the required amount of dilutor. For the first insemination 151 ewes were inseminated with sperm diluted  $\times 10$  and the remaining fifty-one with sperm diluted  $\times 2$  and  $\times 4$ ; for succeeding inseminations dilutions of  $\times 2$  and  $\times 4$  were used.

The instruments used for insemination were disinfected in the following manner: the speculum was swabbed with 65 per cent alcohol

and dried thoroughly with sterile cotton-wool swabs. After each ewe the speculum was washed in water, if necessary, and then swabbed. A 2-ml. glass syringe and vulcanite nozzle was used for the introduction of the sperm. The syringe was sterilized in a hot-air oven, and the vulcanite nozzle was sterilized by filling it with 65 per cent alcohol which was allowed to remain in it for a few minutes. It was then thoroughly washed out with dilutor. After inseminating a ewe, the outside of the nozzle was disinfected by swabbing with 65 per cent alcohol and dried with sterile cotton-wool swabs.

For the actual insemination the ewe was stood on a table or placed over a broad wooden rail about 3 ft. 6 in. high. The vulvar region was washed with a sponge and water. The speculum was disinfected and inserted into the vagina and the cervix exposed. The cervix was illuminated with a battery lamp fixed to the forehead of the operator. The tip of the vulcanite nozzle was now inserted into the cervix and 0.2 ml. sperm introduced. If the nozzle is introduced too far the pressure of the cervical tissue against the tip of the nozzle will prevent the exit of the sperm, but this can be avoided by withdrawing the nozzle slightly. The type of speculum used is of some importance and it is recommended that the Russian model ewe speculum be used (Holborn Surgical Instrument Co.).

The animals used in this experiment were a flock of 202 "high-grade" Merino ewes, and the ram was a pure-bred Merino. This ram was used for all the ewes.

Insemination was carried out once daily from 18 December 1935 to 29 February 1936.

Four vasectomized and vasoligated rams were used to pick out the ewes on heat. These rams were put with the ewes at 6 a.m. every morning and insemination was usually carried out about 9 a.m. The ewes were under supervision during this period and, in addition, the rams were "keeled" with raddle. Immediately a ewe was served, she was removed from the flock to prevent the ram serving her repeatedly. When a considerable number of the ewes had been inseminated, the number of "teaser" rams was reduced to three and later to two.

## RESULTS

No. of inseminations ...	1	2	3	4
No. of ewes	202	91	34	17
No. of ewes "settled"	111	57	(17)	—
% of ewes "settled"	54.9	62.6	—	—

The number of ewes that "settled" (as indicated by those that did not return to the ram) was 168, i.e. 83.2 per cent for the first two inseminations. Of the thirty-four ewes inseminated for the third time seventeen had returned to the ram by the end of February when the vasectomized rams were taken out. The number of ewes that lambed was 141, and nine ewes which died were found to be pregnant on post-mortem examination. Thus 74.3 per cent of the ewes conceived. The total number of live lambs, dead lambs and foetuses was 188 (93.1 per cent).

#### DISCUSSION

Before the beginning of an insemination season a ram or rams of a suitable type should be selected. These rams should be thoroughly tested for sexual vigour and sperm production. Rams vary considerably in sexual vigour and in the ease and rapidity with which they serve a ewe. Sexual vigour can be judged by observing the behaviour of a ram when with an oestrous ewe, e.g. when attempting to collect sperm with the artificial vagina. A ram when presented with a ewe after a long period of rest may be over-excited, and may not experience a proper or complete ejaculation. Several examinations should therefore be made on such rams. The volume of the sperm produced, the physical characters, motility and density must be determined. The average volume of sperm produced by the ram is said to be about 1.6 ml., but in the present experiment the average volume was approximately 0.5 ml. The quality of the sperm can be readily determined by naked-eye examination. A good specimen of sperm is of a creamy colour and of thick consistence. Motility can be noted in a good specimen without recourse to a microscope, but it is advisable in all cases to make a microscopical examination as well. If this is done immediately after collection of the sperm the motility can be judged on an ordinary slide examined, but it is best to use a hanging drop preparation since sperm dries up rapidly with loss of motility at room temperature (18–20° C.). Sperm diluted for use should also be examined before and, if possible, after use. This is of particular importance when a new dilutor is being used. In one instance a dilutor used by the author seemed normal when the diluted sperm was examined before the insemination was carried out, but it had killed all the sperm after the lapse of half an hour. This examination is recommended as a routine procedure, but with a dilutor that has been thoroughly tested it should not be necessary. The density of the sperm can, as has been mentioned, be determined from its physical appearance, and this is confirmed by the microscopical examination. The technique

for a sperm count has not been fully investigated by the author but, using a haemocytometer and a dilution of  $\times 200$ , the average for twenty-four specimens from six rams was 2,907,775 per c.mm.

Several lots of dilutor were made with water distilled in a copper still. These dilutors had varying effects on the sperm; some were toxic to varying extents and others again were normal. Water for the preparation of dilutors is now distilled in glass vessels and this toxic effect has been obviated. One lot of dilutor, which was filled into test-tubes from an automatic pipette with rubber junctions, also proved toxic. This toxic effect does not arise if a glass burette is used.

Since in many cases on account of the unsuitable type of speculum used sperm could not be injected directly into the cervix, the results obtained in this experiment cannot be regarded as an indication of what might be expected using a suitable speculum and introducing the sperm directly into the cervix. A suitable speculum was used for the third and fourth inseminations but the percentage conception after the third insemination is at the most 50 per cent. However, this group of thirty-four ewes doubtless contained a number that had failed to conceive previously on account of sterility.

It is known that the introduction of sperm into the vagina gives only half the percentage of conceptions as the same quantity introduced into the cervix. The injection of 1 ml. of sperm into the vagina is considered to be the equivalent of 0.2 ml. into the cervix. In the present experiment the injection of sperm into the vagina, instead of into the cervix in a number of cases, is considered to be, at least to some extent, the reason for the low percentage of conceptions following the first insemination in the present experiment. Where possible in these cases larger quantities of sperm were injected into the vagina but it was seldom that sufficient sperm was available to permit of the injection of 1 ml.

The dilutor GPS-2 in a dilution of  $\times 8$ , used in mass work, has given a percentage of fertilization of from 65-80 (Milovanov & Selivanova, 1932). In actual practice a dilution of  $\times 8$  seems to be the optimum for this dilutor. Kuznetzova (cited by Milovanov & Selivanova, 1932) gives the following data on the percentage of "settling" in 263 ewes with various dilutions of GPS-2.

Degree of dilution	0	2	4	8	16	32	64
% of "settling"	79.2	80.0	80.0	83.7	59.2	62.0	59.0

The Merino is not highly fertile compared with other breeds of sheep. In Australia it is said that the lambing percentage in the vast

majority of flocks is well under 100, and it rarely exceeds 90. During unfavourable seasons it may fall to under 50 and even less (Gilruth, 1936). These figures are estimates from counts made at marking. The actual lambing percentage would certainly be greater. In the South African Merino fertility is also comparatively low, probably not exceeding 90 per cent (Quinlan *et al.* 1932). The Kenya Merino is a "high-grade" sheep that has been developed by crossing local Masai ewes with imported pure-bred Merino rams. Nothing is known of the fertility of Masai sheep and there are no accurate records of fertility in the high-grade Merino flocks. In the larger Merino flocks in Kenya, numbering perhaps 5000–10,000 breeding ewes, 80 per cent is considered a good lamb crop. This again is merely an estimate made at "tailing". The lambing percentage in the experimental group of 202 ewes that were artificially inseminated therefore compares quite favourably with that after natural mating.

The ewes in this experiment were inseminated once daily, and probably therefore comprised ewes at all stages of heat. It is necessary to know if insemination at the beginning of heat is as effective as insemination at the end of heat. This question is related to that of the vitality of spermatozoa in the female genital tract, and with the time of ovulation in relation to the duration of oestrus. The Russian view is that one insemination from the beginning of heat to 30 hours is adequate (Milovanov, 1934). The work of Quinlan *et al.* (1932, 1933), who investigated this problem from a different viewpoint, namely vitality of spermatozoa in the female genital tract, is in agreement with this Russian view. These workers state that the cervical canal appears to be the natural habitat of the sperm from which small numbers are continually passing forward to the uterus and Fallopian tubes. Some living sperms may be found in the cervical canal up to 48 hours after service, and 24 hours after service sperms are still numerous and actively motile.

The experimental determination of the optimum time of artificial insemination has been investigated by different workers. The percentage conception using dilutor GPS-2, with insemination carried out at the following intervals from the beginning of heat, namely, 2–18, 18–26, 26–42, 30–46, 42–50 and 46–54 hours, was 80·99, 84·90, 48, 38·65, 14·5 and 2·6 respectively (Kardymocic *et al.* 1934). Zajac (1935) gives the following results: the percentage conception at 8, 16, 24, 32, 40, 48 and 56 hours after the beginning of heat were 70·2, 82·5, 85·8, 82·9, 76·9, 66·7 respectively. The optimum time for insemination in the first experiment was 18–26 hours after the beginning of heat, and in the second 24 hours, though by the thirty-second hour the percentage of conceptions (82·9)

was still high. Observations on the duration of oestrus in high-grade Merino sheep in Kenya so far indicate that the majority of heat periods are of less than 30 hours' duration. It would therefore appear that a single insemination during a heat period would be effective in these sheep, but this question will require further investigation.

#### PRACTICAL APPLICATIONS

The practical applications of artificial insemination to livestock breeding are too well known to require more than a brief mention here. In particular, however, it is desired to indicate briefly the application and value of artificial insemination of sheep in Kenya and other colonies.

The application of artificial insemination to sheep breeding in Kenya would benefit both the European farmer and the native. In Kenya 3 per cent is the usual percentage of rams in European-owned flocks. In the larger flocks it is impossible to provide anything approaching this number of pure-bred rams, and consequently a large number of grade rams have to be used. If artificial insemination were used, grade rams could be entirely dispensed with and the number of pure-bred rams required could be considerably reduced. Moreover, a much better type of ram could be used than is at present possible. On the Experimental Station, Naivasha, eighteen rams were kept for a flock of about 600 ewes. Of these rams eleven were pure-bred and seven "grades". In future it is intended to keep one pure-bred ram of a very much better type than is at present available for this flock of ewes, which with the addition of 300 native sheep will number approximately 900 ewes. This number of ewes does not by any means represent the maximum number that can be inseminated with one ram. A Rambouillet ram has sired in one season a crop of 2580 lambs (Parsutin, 1934). Two other specially selected rams have sired 2733 and 1403 ewes respectively during a mating season with over 70 per cent of fertilization (Kuznetzova, 1932).

Native sheep are of small value, averaging probably about 5s. per head in Kenya. They supply at present a purely local demand for slaughter. In the event of an export trade for mutton being developed there is the possibility of crossing these sheep with rams of mutton breeds, but this would have to be carried out in relation to the available plane of nutrition. There would seem to be a greater possibility of developing a cross-bred native wool-bearing sheep. It is known that "grading" Masai ewes with Merino rams results in a good wool type of sheep. The improvement of sheep in the Masai Reserve would now seem to be a practical proposition. In the 1931 *Report* of the Kenya Depart-



ment of Agriculture it is estimated that there are a few million native-owned sheep in the Northern Frontier Province. This is a very dry area, and would probably be well suited to a cross-bred Merino sheep. A flock of 200 Masai ewes, and 100 Black-headed Persian ewes which are found in the Northern Frontier Province have been purchased for this station, and it is proposed to cross these sheep with a pure-bred Merino ram in order to determine the wool characteristics of succeeding generations. In time, therefore, the potential value of such cross-bred sheep will be fairly well known.

#### SUMMARY

An account is given of a preliminary experiment on artificial insemination of cross-bred Merino sheep in Kenya. 202 ewes were inseminated and 74.3 per cent conceived. The practical application of artificial insemination to sheep breeding in Kenya is discussed.

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## GRASS SILAGE

A COMPARISON OF THE CHANGES INVOLVED IN THE  
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## INTRODUCTION

THE object of this communication is to give an account of an experiment, carried through in the autumn and winter season of 1934-5, designed to determine the changes in composition involved in making silage by the ordinary, molasses and A.I.V. processes. The changes in composition included those of both crude as well as digestible nutrients.

*Grass*

A suitable area (15 acres) from which hay had been taken earlier in the year was set aside in September and dressed with 1 cwt. of a nitrogenous artificial manure. In mid-October 1934, when the grass was 4-6 in. high cutting and filling commenced; concurrently sample quantities were despatched to Jealott's Hill Experimental Station for digestibility determinations.

*Silos*

The three experimental silos, consisting of bolted wood stave cylinders 9 ft. 6 in. in diameter and 5 ft. deep, were sunk to a distance of  $2\frac{1}{2}$  ft. into the ground and had a grass capacity of approximately 9 tons. Each was provided with a detachable over-silo of similar dimensions, bolted to the lower one at the time of filling. This over-silo was removed a few days after filling when the material had sunk into the lower section. The silo intended to receive the acid-treated grass was provided with a sump and drain.

*Filling. Ordinary silage*

Weighed loads of freshly cut grass were trodden well into the silo with over-silo attached. At a point when the silo was half filled an overnight period (of about 12 hours) was allowed to permit the mass to heat up to 80° F. The filling was completed on the following day when the

material nearly reached to the top of the over-silo. A covering of sacks was then laid on the surface and earth heaped over for the twofold purpose of excluding air and pressing the material into the under-silo. When the material had sunk sufficiently (7-9 days) the over-silo was removed and more earth heaped over to bury the silo completely.

#### *A.I.V. silage*

The method of filling was similar to that employed for the ordinary silage with the exception that additions of acid to successive grass layers were made from a watering-can at the rate of about 15 gallons per ton of fresh grass. The A.I.V. acid consisted of HCl with some  $H_2SO_4$ <sup>1</sup> diluted to give a 2*N* solution. The actual quantity used was decided by a predetermination when green grass was ground and titrated electrometrically with varying quantities of acid. Extrapolation to a pH of 3.5 indicated the precise amount to be used. No particular difficulty was experienced in filling, the only precautions being necessary were in wearing rubber boots for treading. The possibility of rapid mould formation on the surface was guarded against by spraying with a suspension of mustard oil. Earthing over was carried out as in the case of the ordinary silage. The addition of considerable quantities of liquid necessitated the provision of a drain and sump and several times in the weeks following it was found necessary to empty the system. Filling was continuous and no interval was allowed for the mass to heat.

#### *Molassed silage*

The filling operations involved were precisely similar to those employed in the ordinary except that each successive 6 in. layer of grass was sprayed with a solution of molasses at the rate of about 2 lb. for each 100 lb. of grass. For this purpose the requisite weight of molasses was diluted with twice its volume of water. It was presumed that the added molasses would provide a source of readily fermentable carbohydrate material and thus assist in producing a supply of lactic acid thereby conserving the soluble carbohydrate constituents of the grass.

#### *Sampling*

*Grass.* From each load of grass, as it was being fed into the silos, ten to a dozen subsamples were taken, bulked, stored in airtight tins and forwarded to the laboratory for analysis. The bulk sample for each load was kept separate.

<sup>1</sup> Virtanen, A. I. *Acta chem. fenn.* (1933), A, 6.

*Silage.* Sampling of the silage was carried out by a similar method when the silos were being emptied on to tared carts.

On reaching the laboratory the samples from individual loads were separately brought to air-dry condition and later composited on the basis of load weight for further determinations. Simultaneously with emptying a silo two separate 2 cwt. cubes of silage were cut and removed intact, one from about one-third of the way down on one side of the silo, and the other from about two-thirds down on the other side. These were forwarded to Jealott's Hill for digestibility determinations. This method of sampling applied to all three treatments.

#### *Emptying the silos*

In March 1935 all three silos were emptied. The material from each particular silo was transferred to tared carts and then placed in an over-silo erected at a point convenient to the animals to which it was ultimately fed. The emptying process had of necessity to be carried out rapidly since exposure before weighing for any considerable period would have resulted in additional changes in composition and the growth of moulds, in fact the emptying was actually carried out in one day in order to obviate such changes.

#### *Analytical methods*

*Dry matter.* This was determined in two stages, first by subjecting the material in 150 g. lots to a current of air at 80–90° C. and subsequently allowing it to attain an air-dried condition. Secondly, by drying a subsample of the air-dried matter in a steam oven for absolute dry-matter estimation.

#### *Crude nutrients and pepsin-HCl soluble protein*

These constituents were determined on the air-dry material and later corrected to a dry-matter basis. The usual conventional methods were employed; for the true protein precipitation, trichloroacetic acid, and for the pepsin-HCl soluble protein, Wedermeyer's method.

#### *Volatile acids, bases and amino acids*

The volatile acids and bases and the amino acids were determined by Foreman's method.<sup>1</sup>

*The pH determinations* were made with the quinhydrone electrode.

*The minerals,*  $P_2O_5$  and  $CaO$ , were determined by conventional

<sup>1</sup> *Biochem. J.* (1920), **14**, Nos. 3 and 4, pp. 451–73.

methods (volumetrically by a modified Pemberton-Neuman method and titration with potassium permanganate).

*Composition and changes of the ingoing grass and resultant silage*

Table I gives the composition of the grass used and the silage resulting in terms of crude nutrients,  $P_2O_5$  and  $CaO$ .

Table I

	Ordinary			Molasses			A.I.V.		
	Silage out			Silage out			Silage out		
	Un-			Un-			Un-		
	Grass in	corrected	Corrected	Grass in	corrected	Corrected	Grass in	corrected	Corrected
	%	%	%	%	%	%	%	%	%
Ether extract	3.39	6.84	9.55	3.88	5.56	8.28	3.24	5.00	6.84
Ash	9.48	8.94	8.55	9.65	10.12	9.68	9.59	8.02	7.84
Crude fibre	28.63	28.68	27.43	27.18	27.14	25.97	27.67	26.02	25.42
Crude protein	16.02	15.16	15.85	15.43	15.33	16.02	15.34	16.00	16.06
N-free extractives	42.48	40.38	38.62	43.86	41.85	40.05	44.16	44.27	43.24
Organic matter	90.52	91.06	91.45	90.35	89.88	90.32	90.41	91.98	92.16
True protein	12.62	8.08	7.73	12.94	8.26	7.90	12.90	12.24	11.96
$CaO$	0.79	0.93	0.89	0.82	1.01	0.97	0.81	0.71	0.69
$P_2O_5$	0.85	0.74	0.71	0.82	0.71	0.68	0.78	0.78	0.76
Ratio	True protein			Crude protein					
	0.79	0.53	0.49	0.84	0.54	0.49	0.84	0.74	0.72

The silage constituents are shown both uncorrected and corrected for volatile acids and bases presumed to have been lost during the drying process.

The figures show changes of the usual order. There is an increase in ether extract which is due largely to the organic acids resulting from the silage process. The A.I.V. silage shows the lowest increase and the ordinary silage the greatest. The only change to be seen in the ash is found in the A.I.V. silage, which is also reflected in the calcium content of this material.

The main differences are seen in the content of true protein and it is noticeable that in this regard the A.I.V. process has been the most efficient in preventing protein breakdown.

*Acidity (pH), volatile constituents and amino acids*

Table II shows the pH of the fresh grass and the silage resulting from the three processes. It will be noted that there was only slightly higher acidity in the ordinary product than that found in the grass. The highest degree of acidity (part as a result of acid added) was found in the A.I.V. product (3.6) and the molasses appeared midway (4.4).

The volatile constituents and the amino acids (expressed as acetic acid and protein) are shown as percentages of the fresh grass and silage.

If desired they can be calculated on the dry matter by employing the figures given for that constituent. No estimate of the volatile acids, bases and amino acids was made on the dried silage, on the assumption that the losses were complete during the drying process. This assumption may not be strictly valid.

Table II

	Fresh grass	Ordinary silage	Molasses silage	A.I.V. silage
pH	5.6	5.3	4.4	3.6
Volatile acids ( $\equiv$ acetic acid %)	0.32	0.70	0.68	0.43
Volatile bases ( $\equiv$ protein %)	0.05	0.31	0.31	0.10
Amino acids ( $\equiv$ protein %)	0.06	0.27	0.28	0.31
Dry matter (%)	21.9	18.4	20.8	19.8

Although the content of volatile acids was used to correct the ether extract fractions shown in Table I such was not taken into account when calculating the starch equivalents given in Table IV. This point is of importance since it would be unfair to attach the same feeding value to acetic acid as that part of the fraction directly extracted.

In the silages referred to in this paper the quantity of volatile constituents and amino acids formed during ensiling was small.

### *Digestibility*

Samples of grass were taken and sent to Jealott's Hill for digestibility trials during the process of filling the silos. Also, as previously stated, samples of silage in undisturbed cakes, each weighing approximately 2 cwt., were similarly forwarded from each silo. These determinations were carried out on sheep by the method which has been described elsewhere.<sup>1</sup> In these trials the intake was at the maintenance level and therefore somewhat low in all cases. The ordinary and molasses silage were definitely palatable and the sheep would probably have eaten larger amounts. The A.I.V. silage proved less palatable which may have resulted in the animals picking out the leafier portions and neglecting the stalks.

### *Digestibility coefficients*

Table III shows the digestibility coefficients for the various nutrients in both fresh grass and silage.

In the instance of the A.I.V. silage a second digestibility trial was conducted which gave figures for digestible nutrients slightly lower than

<sup>1</sup> Watson, S. J. & Horton, G. A. *Emp. J. exp. Agric.* (1936), 4, 25.

in the first trial. The results shown in Table III (last column) are the average of both determinations.

Table III

	Fresh grass	Ordinary	Silage	
			Molasses	A.I.V.*
Dry matter	67.8	60.3	64.3	68.0
Organic matter	70.7	64.0	67.7	71.7
Ether extract	47.8	65.7	62.1	57.5
Crude fibre	70.8	72.1	74.7	73.9
N-free extractives	71.4	58.8	66.0	71.9
Crude protein	72.3	62.2	62.1	72.0
True protein	68.2	33.3	40.3	64.0
Daily intake of dry matter during trials (g.)	994	663	685	445

\* Average of two trials.

Table IV gives the percentage of digestible nutrients in the grass and silage, together with the starch and protein equivalents. These have been calculated from the values for composition in Table I and the digestibility coefficients in Table III.

Table IV

	Ordinary		Molasses		A.I.V.	
	Grass	Silage	Grass	Silage	Grass	Silage*
Dig. ether extract	1.62	6.27	1.85	5.14	1.55	3.93
Dig. crude fibre	20.27	19.78	19.24	19.40	19.59	18.79
Dig. crude protein	11.58	9.86	11.16	9.95	11.09	12.00
Dig. N-free extractives	30.33	22.71	31.32	26.43	31.53	31.09
Dig. true protein	8.61	2.57	8.83	3.18	8.80	7.65
Starch equivalent	52.1	47.1	53.2	49.4	52.9	55.8
Protein equivalent	10.10	6.22	10.00	6.57	9.95	9.83

\* Average of two trials.

Table V shows balance sheets for the crude and digestible nutrients for the three silos.

## DISCUSSION OF RESULTS

### *Changes in gross and digestible nutrients*

Since the changes brought about are mainly those of fermentation character, losses may be expected, and in fact, occur in all the main nutrient constituents both crude and digestible.

An exception was found only in the ether extract when an increase was shown, largely due, it may be assumed, to the appearance of acid bodies resulting from the breakdown of carbohydrates and proteins.

In one instance, molasses silage, an increase of the CaO in the

Table V

	Grass in		Silage out		Difference			
	Crude	Digestible	Crude	Digestible	Crude		Digestible	
					lb.	%	lb.	%
Ordinary silage								
Dry matter	3514.1	2382.6	2666.0	1607.6	-848.1	-24.1	-775.0	-32.5
Ether extract	119.0	56.9	182.3	119.8	+ 63.3	+53.2	+ 62.9	+110.5
Fibre	1006.0	712.2	764.7	551.3	-241.3	-24.0	-160.9	-22.6
Ash	333.0	—	238.3	—	- 95.0	-28.5	—	—
Crude protein	562.9	407.0	404.1	251.4	-158.8	-28.2	-155.6	-38.2
N-free extractives	1492.9	1065.9	1076.6	633.0	-416.3	-27.9	-432.9	-40.6
Organic matter	3181.1	2249.0	2427.7	1553.7	-753.4	-23.7	-695.3	-30.9
True protein	443.5	302.5	215.3	71.7	-228.2	-51.5	-230.8	-76.3
CaO	27.8	—	23.7	—	- 4.1	-14.7	—	—
P <sub>2</sub> O <sub>5</sub>	29.9	—	18.9	—	-11.0	-36.8	—	—
Starch equivalent	—	1830.8	—	1167.7	—	—	-663.1	-36.2
Protein equivalent	—	354.9	—	165.8	—	—	-189.1	-53.3
Molasses silage								
Dry matter	*3984.3	2701.4	3484.4	2240.5	-499.9	-12.5	-460.9	-17.1
Ether extract	143.2	68.4	193.8	120.3	+ 50.6	+35.3	+ 51.9	+75.9
Fibre	1004.2	711.0	945.7	706.4	- 58.5	- 5.8	- 4.6	- 0.6
Ash	382.0	—	352.7	—	- 29.3	- 7.7	—	—
Crude protein	581.7	420.6	534.1	331.7	- 47.6	- 8.2	- 88.9	-21.1
N-free extractives	1873.2	1337.5	1458.1	962.3	-415.1	-22.2	-375.2	-28.1
Organic matter	3602.3	2546.8	3131.7	2120.2	-470.6	-13.1	-426.6	-16.8
True protein	483.2	329.5	288.0	116.1	-195.2	-40.4	-213.4	-64.8
CaO	32.7	—	33.8	—	+ 1.1	+ 3.4	—	—
P <sub>2</sub> O <sub>5</sub>	32.7	—	23.7	—	- 9.0	-27.6	—	—
Starch equivalent	—	2119.6	—	1609.8	—	—	-509.8	-24.0
Protein equivalent	—	398.4	—	228.9	—	—	-169.5	-42.5
A.I.V. silage								
Dry matter	4282.5	2903.5	3507.1	2518.1	-775.4	-18.1	-385.4	-13.3
Ether extract	139.0	66.4	178.7	108.6	+ 39.7	+28.6	+ 42.2	+63.6
Fibre	1184.6	838.7	912.5	695.3	-272.1	-23.0	-143.4	-17.1
Ash	410.7	—	281.3	—	-129.4	-31.5	—	—
Crude protein	657.1	475.1	582.0	438.2	- 75.1	-11.4	- 36.9	- 7.8
N-free extractives	1891.1	1350.2	1552.6	1169.1	-338.5	-17.9	-181.1	-13.4
Organic matter	3871.8	2737.4	3225.8	2412.9	-646.0	-16.7	-324.5	-11.9
True protein	552.5	376.8	429.2	291.0	-123.3	-22.3	- 85.8	-22.8
CaO	34.7	—	24.2	—	- 10.5	-30.3	—	—
P <sub>2</sub> O <sub>5</sub>	33.4	—	26.7	—	- 6.7	-20.1	—	—
Starch equivalent	—	2265.4	—	1974.5	—	—	-290.9	-12.8
Protein equivalent	—	426.1	—	362.2	—	—	- 63.9	-15.0

\* Including the weight of added molasses.

fermented material over that in the fresh grass occurred which was probably insignificant. The extent of the changes varied in each process and is analysed below.

### *Ether extract*

Gross gains of the nutrient appeared to be highest in the ordinary silage (53.2 per cent), lowest in A.I.V. (28.6 per cent), the molasses material falling midway (35.2 per cent). A similar order was followed in the digestible ether extract, viz. ordinary silage (110.5 per cent), A.I.V. (63.6 per cent), and molasses (75.9 per cent). It should be noted that corrections for gains of volatile acids (presumably lost in drying) appear in this fraction enhancing the uncorrected figures.



*Fibre*

In the crude fibre, losses in the ordinary (24 per cent) and A.I.V. materials (23 per cent) were very similar. A more definite reduction of loss of fibre obtained as a result of spraying with molasses (5.8 per cent). In the digestible fibre the loss on the untreated silage (22.6 per cent) was of an order similar to that found in the crude, somewhat lower in the A.I.V. (17.1 per cent), and of negligible dimensions in the molasses (0.6 per cent). It would appear, therefore, that the saving of this particular nutrient was most pronounced in the molasses product where the fermentation processes had doubtless made use of the added carbohydrate.

*Crude protein*

Regularity in the losses of this constituent were not so evident as in the case of the fibre. While a heavy loss fell on the crude protein of the ordinary product (28.2 per cent), a much heavier burden fell on the digestible fraction (38.2 per cent). This was also the case in the molasses produce (crude 8 per cent and digestible 21.1 per cent) but occurred at a lower level.

The protective action of the added acid was shown up by the losses in the A.I.V. material where those on the crude were 11.4 per cent, and on the digestible side 7.8 per cent.

*True protein*

The results for the crude protein losses were reflected even more strongly in the true protein. In the ordinary product the losses in the crude true protein were 51.5 per cent, and on the digestible 76.5 per cent, both of which may be described as enormous. Treatment with molasses reduced the losses somewhat, 40.4 and 64.8 per cent respectively. The effect of the acid treatment was undoubtedly favourable in reducing the losses which were approximately 22 per cent for both the total and digestible fractions.

This fact is also seen in the alternative expression of the nitrogen as protein equivalent. Stated as such the greatest loss occurred in the ordinary silage (36.2 per cent), followed by a considerably lower loss in the molasses product (24 per cent), and the lowest in the A.I.V. (12.8 per cent).

*Nitrogen-free extractives*

The greatest loss in this nutrient is seen in the ordinary silage where that in the digestible fraction (40.6 per cent) is considerably higher than that in the total (27.9 per cent). Again the molasses product (crude 22 per cent

and digestible 28.1 per cent) falls midway and the A.I.V. at the lowest end of the scale (crude 17.9 per cent and digestible 13.4 per cent). It is worthy of note that the acid treatment has reduced the losses in the digestible N-free extractives compared with those in the crude fraction, a condition which is reversed in both the other treatments.

#### *Other constituents*

*Ash.* Losses in the crude ash content occurred during the making of all three forms of silage but in variable quantities. The lowest loss appeared in the molasses product (7.7 per cent), the highest in the A.I.V. (31.5 per cent), that in the ordinary falling midway (28.5 per cent). At first it was thought that these results may be associated with the pH of the products, but this does not seem to be the explanation since the molasses silage was actually more acid than the ordinary. The CaO showed a loss in the ordinary (14.7 per cent) and A.I.V. products (30.3 per cent) and a slight but probably insignificant gain in the molasses silage (3.4 per cent). The  $P_2O_5$  also showed a loss in the ordinary (36.8 per cent), molasses (27.6 per cent) and A.I.V. (20.1 per cent). It should be noted that small quantities of CaO (1 lb.) and  $P_2O_5$  (0.1 lb.) were added in the molasses.

#### *Dry matter*

The dry-matter changes may be regarded as useful all-round criteria whereby to judge efficiency of the silage-making processes. Inspection shows the greatest loss of crude dry matter in the ordinary product (25.1 per cent), the least in the molasses (12.5 per cent), with the A.I.V. in an intermediate position (18.1 per cent). Examination of the losses of digestible dry matter places the last two products in the reverse order (molasses 17.1 per cent and A.I.V. 13.3 per cent), the ordinary still standing highest (32.5 per cent).

While the heaviest losses in the dry matter would appear to fall on the digestible fraction in the molasses and ordinary silage, such is not however the case in the A.I.V. product. This would apparently be due to the conservation of the protein, particularly the pure protein.

#### *Starch equivalent*

An alternative criterion of efficiency of the ensiling process may be sought in the losses of feeding material stated as starch equivalent. Here the greatest losses fall on the ordinary product (36.2 per cent). This is followed by the molasses (24.0 per cent) with the A.I.V. again lowest

(12·8 per cent). Again the acid process would appear to be the most effective in conserving the digestible part of the material.

#### GENERAL CONCLUSIONS

##### *Palatability and keeping quality*

All three products were palatable to cattle. Such was not the case with the sheep used in the digestibility trials, where there was some difficulty in persuading the animals to eat up the A.I.V. silage. No losses due to moulds were experienced in any of the three products in making. This was doubtless due to the considerable care taken in packing and sealing which shows that with this type of silo almost ideal conditions can be obtained. Under the conditions of this experiment where each silo had to be emptied completely at one operation certain difficulties arose in not being able to use up the materials as rapidly as would have been desired. It was noted that whereas the ordinary silage rapidly developed moulds before it could all be fed to the cattle, such was not the case in the A.I.V. and molasses products.

##### *Composition*

The outstanding changes in composition were the increases in the ether extract—partly due to the corrections applied—and losses in all other fractions. Variations due to methods employed were considerable, viz. improved conservation of proteins in the A.I.V. process and of carbohydrates (including fibre) in the molasses process.

##### *Digestibility*

The order of digestibility based on dry-matter content places the ordinary silage lowest (60·3 per cent), the A.I.V. highest (71·8 per cent), and the molasses intermediate (64·3 per cent). In this respect the A.I.V. material possibly shows up in a rather too-favourable light since there was considerably greater difficulty in getting the sheep to eat it, at any rate as readily as the molassed and ordinary silage. Being less palatable there was a tendency for the sheep to choose the leafy parts of the silage. In a second trial on A.I.V. material less food was offered to ensure completion of ration and somewhat lower digestibility coefficients obtained.

##### *Losses*

Judged on the basis of crude dry matter the molasses silage shows the lowest losses, the ordinary the highest, and the A.I.V. holding an inter-

mediate place. The loss of true protein was considerably lower in the A.I.V. material than in the others. The added molasses appear to have exerted a protective action on the plant carbohydrates by supplying readily fermentable material.

The losses of digestible nutrients and starch equivalent were least in the A.I.V., highest in the ordinary, and intermediate in the molasses product.

The heavy losses in the true protein in the ordinary and molasses materials may, in practice, be rather less serious than the figures suggest since the non-protein nitrogenous products may have some feeding value or at any rate a protein-sparing value.

### *Application to farm practice*

Of the three processes the A.I.V. presents more difficulties in farm practice than the other two. These are associated with the considerable danger in dealing with strong acids, which certainly demand more skill and experience in handling than is likely to be found on the average farm. On the other hand, no such difficulty is apparent in the use of molasses. There appears, therefore, every reason to encourage the use of molasses both for the reason stated and even further since it is attended by the lowest loss in dry matter.

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# STUDIES IN TROPICAL SOILS

## IV. ORGANIC TRANSFORMATIONS IN SOILS, COMPOSTS AND PEAT

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### I. INTRODUCTORY

MOST of the earlier investigations of soil organic matter were concerned with the transformations that proceed under temperate climate conditions when vegetable residues or green manures are applied to soils. The results have led to the generally accepted view that the added organic matter ultimately gives rise to "resistant humus", having a carbon to nitrogen ratio of approximately 10, indicating loss of cellulosic substances and the gradual accumulation of nitrogenous compounds. It is assumed by some investigators that much of the residual protein present in humus occurs as microbial material synthesized in the bodies of the micro-organisms involved in these organic transformations.

The degree of breakdown of organic matter added to soil may therefore roughly be gauged by the C/N ratio, and recent researches in temperate countries have indicated that the value diminishes significantly with depth of soil within a profile. Such changes have been noted by McLean<sup>(1)</sup> for two Welsh soil profiles, in which the lowest value for C/N ratio was found to be 10.8, approximating to the theoretical ultimate value of 10.0. Within tropical soil profiles, such as those studied by Hardy *et al.* in the West Indies region<sup>(2)</sup>, the C/N ratio may range from over 10 in the surface soil to less than 5 at depths below 2 ft. In many cases, the ratio is considerably less than 10 for all layers of the profile. For the cacao soils of Trinidad, Tobago and Grenada, the yield of cacao appears to be positively correlated with the C/N ratio<sup>(3)</sup>, and not necessarily solely with the total amount of organic matter present in the surface soil rich in nutrients and containing the bulk of the "feeding roots".

In order to explain these and other observations, studies of organic transformations under tropical conditions were made by the writer for

some typical examples of compost manures and West Indian soils, by applying the well-known fractionation methods of Waksman. The aim of Waksman's scheme of proximate quantitative organic analysis is to separate the different chemical components of soil organic matter into seven more or less arbitrary groups. In the investigations described below, a modified scheme of analysis, similar to that recently proposed by Waksman & Stevens<sup>(4)</sup>, has been employed. The following fractions were estimated:

(i) *Benzol-alcohol extractives*. Organic matter soluble in a boiling mixture of equal parts of benzol and ethyl alcohol.

(ii) *Hydrolysable protein*. Nitrogenous compounds (calculated as protein) rendered soluble by treatment with 78 per cent sulphuric acid for 2 hours, followed by dilution with water to 5 per cent concentration of sulphuric acid, and boiling for 6 hours.

(iii) *Hemicelluloses and celluloses*. Carbohydrate substances (calculated as cellulose ( $C_6H_{10}O_5$ )), rendered soluble by hydrolysis in the last estimation. Reducing sugars were determined in the filtered extracts by the method of Ling & Carter<sup>(5)</sup>, and the results multiplied by the usual factor, 0.9.

(iv) *Resistant protein*. Nitrogenous compounds present in the residue after hydrolysis, calculated as protein.

(v) *Lignin humus*. Carbon compounds present in the hydrolysed residue, determined by measuring its total carbon content, and subtracting the carbon content of resistant protein. Lignin was calculated from the difference by multiplying by the factor, 100/62, as suggested by Waksman. Determinations of carbon contents were made by the Watts's wet combustion method, corrected by the factor, 1.33<sup>(6)</sup>, or by the Robinson method<sup>(7)</sup>, depending on the quantity of organic matter present in the initial material.

In Waksman's original method the results are expressed as percentages of the total organic matter content, derived by multiplying carbon percentages by 1.724. His recoveries vary from 80 to 101 per cent for plant material. For the organic manures and tropical soils examined by the writer, recoveries range from 77 to 110 per cent, owing to varying dilution by mineral substances. In order to render results more easily comparable, the procedure suggested by Morgan & Lunt<sup>(8)</sup> has been adopted, by which the different fractions are expressed as percentages of "total recovered organic matter", obtained by adding together the amounts of the different groups of substances actually estimated.

## II. EXPERIMENTAL

(A) *Hydrolysis of nitrogenous components*

For the purpose of differentiating between "hydrolysable protein" and "resistant protein", acid hydrolysis is employed by Waksman, and it is assumed that a period of 6 hours' duration is sufficient for complete separation. In order to test this assumption, a number of samples of organic materials and soils were subjected to intermittent hydrolysis with boiling 5 per cent sulphuric acid for hourly periods up to 24 hours. The nitrogen contents of the series of filtrates obtained were then determined. The following samples were used:

- (1) Fresh maize straw.
- (2) Maize straw composted for  $7\frac{1}{2}$  months.
- (3) Fungus mycelium (*Polyporus microporus*), obtained from a decaying forest tree in Trinidad.
- (4) Black calcareous clay sugar-cane soil; surface  $1\frac{1}{2}$  in. layer, Ste Madeleine, Trinidad.
- (5) Subsoil of last, 12-18 in. depth.
- (6) Acidic clay cacao soil; surface  $1\frac{1}{2}$  in. layer, Brasso, Trinidad.
- (7) Subsurface soil of last, 3-6 in. depth.
- (8) Subsoil of last, 6-12 in. depth.

The results of the successive hydrolyses (percentages of total nitrogen rendered soluble, and nitrogen contents of unhydrolysable residues) are presented in Table I.

Table I. *Progressive hydrolysis of organic materials and soils*

Time hours	% of total N removed			Trinidad calcareous soil		Brasso clay		
	Fresh maize straw	Maize compost $7\frac{1}{2}$ months	Fungus	0- $1\frac{1}{2}$ in.	12-18 in.	0- $1\frac{1}{2}$ in.	3-6 in.	6-12 in.
1	59.0	44.8	60.6	35.8	35.5	35.4	35.3	30.5
1-3	17.3	16.2	9.7	24.4	25.6	24.5	16.5	13.4
3-6	5.8	6.6	5.5	16.8	15.6	10.7	13.0	10.9
6-9	3.0	3.1	2.6	7.3	7.0	4.9	6.2	4.4
9-12	2.6	1.4	3.4	5.0	4.5	3.7	3.1	3.5
12-18	2.2	2.4	3.5	4.1	7.7	4.2	6.2	6.0
18-24	2.7	1.5	1.7	2.4	3.5	3.0	4.8	3.5
Total 0-6	82.1	67.6	75.8	77.1	76.7	70.6	64.8	54.8
6-24	10.4	8.4	11.3	18.9	22.7	15.8	20.2	17.4
Residue	15.3	18.0	12.1	10.4	17.6	8.8	25.3	23.6
Total	107.9	94.0	99.2	106.4	116.9	95.1	110.2	95.7

The data in Table I show that the rate of hydrolysis in each case diminishes rapidly after the first hour. In 6 hours the quantity of nitrogen

rendered soluble is approximately seven times as great as that rendered soluble in the subsequent 18 hours.

Evidently, 6 hours' hydrolysis is sufficient to give a reasonably clear partition of the nitrogenous compounds, whilst hydrolysis for one hour effects some differentiation of the more easily hydrolysable nitrogenous compounds. Hydrolysis for 60 hours removed little more nitrogen than hydrolysis for 24 hours. The assumption that 6 hours' hydrolysis is adequate for broad fractionation is confirmed.

The amount of residual unhydrolysable nitrogen varies considerably; it is much higher in the subsoil than in the topsoil. The distribution of nitrogen groups in fungus mycelium is apparently very similar to that of composted maize straw and of soil organic matter, and the view that the nitrogen compounds present in microbial protoplasm occur mainly in the form of acid-resistant protein is not supported by the experimental evidence.

#### (B) *Comparison of tropical and temperate organic materials*

One sample of maize straw, two samples of composts of maize straw, and two samples of Trinidadian black calcareous clay soil were fractionated by the simplified Waksman method, in order to obtain data for comparison with results published by Waksman *et al.* for rye straw composts<sup>(9)</sup> and prairie soil (Waksman & Stevens<sup>(4)</sup>). The data are presented in Table II, in which the experimental values have been recalculated by the procedure suggested by Morgan & Lunt<sup>(8)</sup>.

Table II. *Comparison of tropical and temperate organic materials and soils*

	Total protein	Total celluloses	Lignin humus	Ether-alcohol soluble	Water-soluble
Compost samples					
Fresh maize straw, Trinidad	5.4	49.9	36.8	7.9	—
Fresh rye straw (Waksman)	2.3	74.2	14.7	2.0	6.8
Composted maize straw (107 days)	18.8	35.1	40.8	5.3	—
Composted rye straw (290 days)	18.6	36.5	31.2	2.6	11.2
Composted maize straw (287 days)	33.7	9.7	53.1	3.3	—
Soil samples					
Trinidad topsoil (0-6 in.)	55.9	4.5	39.1	—	—
Fargo soil, "A" horizon (Waksman)	32.9	8.6	31.8	4.0	2.7
Trinidad subsoil (12-18 in.)	87.9	Trace	12.1	—	—
Fargo soil, "B" horizon	31.5	8.9	53.4	3.6	2.6

The samples of compost raw materials (maize straw and rye straw) yielded widely different results, but the partly rotted composted materials gave very similar values. Maize straw composted for 107 days under



open-air conditions in Trinidad (80° F. and 70 per cent relative humidity) is comparable with rye straw composted for 290 days under green-house conditions in Waksman's experiment. After 287 days, the maize straw had reached a much more advanced stage of decomposition than the rye straw composted for 290 days. It contained much less hydrolysable hemicelluloses and celluloses, and more total and resistant protein and lignin humus. Either decomposition is more rapid and profound under the conditions obtaining in Trinidad, or maize straw is more easily decomposed than rye straw. The analytical data support the first possibility, since maize straw initially contains smaller amounts of celluloses and greater amounts of lignin humus than rye straw, and theoretically it should therefore be more resistant to decomposition.

The soil samples also show striking differences. Whilst the surface soils are somewhat similar in composition, the data reveal much smaller amounts of celluloses and lignin humus in the Trinidadian subsoil than in the prairie subsoil from Fargo. The large increase in total protein from topsoil to subsoil is very conspicuous in the Trinidadian soil, but insignificant in the Fargo soil. The explanation of this difference is probably the much higher rate and degree of decomposition occurring under the hot humid conditions of Trinidad.

### (C) *Changes within tropical soil profiles*

In order to study the nature and distribution of soil organic matter occurring in undisturbed natural soil profiles within the hot humid tropics, serial soil samples from three representative West Indian profiles were examined by the simplified Waksman method. The following soil types were studied:

(1) *Black calcareous clay soil.* Petit Morne Estate, Ste Madeleine, Trinidad; grassland. Soil highly fertile to sugar-cane and other crops. Sampling depths: 0-1½, 1½-3, 3-6, 6-9, 9-12 and 12-18 in.

(2) *Red neutral loam soil.* Mandeville, Jamaica; grassland. Soil very infertile to most crops. Sampling depths, as in the last, with the addition of two further 6 in. subsoil layers.

(3) *Red acidic loam soil.* Stony Hill, Jamaica; semi-abandoned banana land. Soil not very fertile. Sampling depths as before, to 24 in.

Laboratory data for the three profiles are presented in Table III; they include soil constants measured by methods customarily employed at the Imperial College of Tropical Agriculture, as well as data derived from Waksman fractionations.

Table III. *Comparison of three tropical soil profiles*

Depth in.	Index of tex- ture	C/N	% organic matter (C $\times$ 1.724)	pH	N %	Hydro- lysable protein	Cellu- loses	Re- sistant protein	Lignin humus
I. Trinidad: black calcareous soil from Petit Morne									
0-1½	62	12.9	10.70	6.5	0.48	22.9	10.3	17.8	49.0
1½-3	56	10.9	7.51	6.9	0.40	28.4	8.4	22.2	41.0
3-6	60	11.2	6.34	7.2	0.33	27.2	11.5	20.8	40.6
6-9	62	9.3	4.31	7.4	0.27	26.4	9.3	23.2	41.0
9-12	53	7.6	2.99	7.4	0.23	41.0	Trace	36.2	22.7
12-18	53	7.3	1.90	7.6	0.15	44.8	Trace	55.2	Trace
II. Jamaica: red soil from Mandeville									
0-1½	41	7.7	4.67	7.1	0.35	29.6	11.2	14.6	44.5
1½-3	37	5.8	3.02	7.1	0.30	36.0	11.5	15.8	36.7
3-6	37	4.6	1.97	7.2	0.24	43.7	1.0	7.6	47.6
6-9	31	4.9	1.42	7.3	0.17	53.5	1.4	5.8	39.4
9-12	34	5.0	0.89	7.2	0.10	63.0	2.1	7.0	27.8
12-18	36	4.4	0.42	7.2	0.06	72.8	3.7	8.0	15.5
18-24	33	5.2	0.40	7.2	0.04	81.0	3.2	11.2	4.5
24-30	34	3.8	0.29	7.1	0.04	70.8	3.5	8.0	17.7
III. Jamaica: red soil from Stony Hill									
0-1½	37	7.9	3.70	7.2	0.27	47.2	7.7	20.4	24.7
1½-3	36	6.1	2.71	6.8	0.26	34.0	0.4	17.8	47.7
3-6	37	5.7	2.31	6.8	0.24	36.3	0.5	17.7	45.5
6-9	34	4.3	1.37	6.7	0.19	45.1	0.6	20.8	33.5
9-12	34	4.0	0.77	6.8	0.11	52.1	1.1	24.0	22.6
12-18	34	5.9	0.69	6.8	0.07	44.4	0.6	33.4	21.6
18-24	37	5.0	0.38	6.9	0.04	44.7	Trace	55.3	Trace

The three soil profiles show great differences in physical and chemical characters, and in the composition of their organic components. The black calcareous Trinidadian clay soil has the highest total organic matter content and C/N ratio, and the different layers of the profile show the least change between the upper and the lower horizons. The Mandeville red soil is richer in organic matter and nitrogen, and is more alkaline in reaction than the Stony Hill red soil, though both show rapid diminution in organic matter and in C/N ratios with depth.

A striking feature of each of the three profiles is the pronounced decrease in C/N ratio with increasing depth. The value of the ratio exceeds 10.0 only in the three uppermost layers (0-6 in.) of the Trinidadian black soil profile; in the other two profiles it is much less than this "critical" value, and in some of the lower layers of the two Jamaican red soils it falls below 4.5.

The Mandeville red soil differs from the other two in that the quantity of resistant protein decreases rapidly with depth, the lowermost layers containing practically all their nitrogen in the form of hydrolysable

protein. The fertile Trinidadian black soil differs from the much less fertile Jamaican red soils in that it contains a considerable quantity of cellulosic substances down to the 9 in. depth, below which celluloses disappear almost completely. The red soils contain appreciable amounts of celluloses only in the shallow surface layers (0-3 or 0-1½ in.), but the lower layers down to the full depth of the profile (2½ or 2 ft.) still contain measurable amounts of cellulosic substances.

In all three cases, high resistant-protein contents and low lignin-humus contents appear to be associated with low contents of celluloses. Possibly, lignin decomposition<sup>1</sup> begins to be effective only when the supply of cellulosic substances has been reduced to a low value. High fertility appears to be associated with a relatively deep penetration of celluloses within the profile. This may explain the fact that an eroded or exhausted agricultural soil, in which much cellulosic organic matter has been removed or lost, recovers but slowly when organic manures or vegetable debris are applied to the surface, since a considerable period of time must elapse before the celluloses derived from the added organic matter can penetrate to appreciable depths (9-12 in.).

The organic matter component of the surface layers of the Trinidadian black calcareous soil and of the Mandeville red soil approximates in composition to the 237 days old maize compost, but that of the Stony Hill red soil exhibits the characteristics of a very much more highly decomposed material, indicating that the surface supply of plant residues is here insufficient to counterbalance the loss of organic matter through decomposition.

The difference between the two Jamaican red soils may be attributed to the fact that one of them is under permanent grass (Mandeville soil), whilst the other (Stony Hill soil) has been under banana cultivation for a considerable period of time. The grass cover in the first appears to have produced a surface layer similar in composition to that of the Trinidadian black calcareous soil, but the absence of a grass cover in the other red soil is associated with a surface layer showing obvious signs of profound decomposition and deterioration. Nevertheless, the Stony Hill red banana soil would probably respond the more quickly to the addition of organic materials, since its lower layers contain relatively large amounts of resistant protein, and seem merely to lack cellulosic substances. Po-

<sup>1</sup> Waksman & Gerretsen<sup>(10)</sup> found that the rate of decomposition of lignin increases rapidly with rise of temperature, a marked difference being recorded between 18 and 27° C. Temperatures in the humid tropics frequently exceed this higher value, so that rapid loss of lignin may occur when plant residues decompose.

tentially, therefore, it appears to be the better of the two red soils, and should prove to possess a greater degree of permanence of fertility.

The application of fractionation methods to the three soil types discussed above has suggested certain relationships between fertility and the composition of the organic matter component which warrant further investigation.

(D) *Further fractionation studies of tropical soil profiles*

For the purpose of further testing certain generalizations suggested in the last section, serial soil samples, taken from five profiles representative of three important cacao soil types occurring in Trinidad, were examined by the simplified fractionation method. Each of the sites is located in a field of known reputation and productivity for cacao. The three additional soil types are described below.

(1) *Chocolate calcareous clay-loam soil* (two profiles). San Salvador Estate and San Pablo Estate, Montserrat District, Trinidad. Rainfall, 100 in. This soil type is regarded as the most fertile soil in Trinidad, and one of the most fertile soils of the West Indies. It regularly supports cacao trees that yield over 16 bags of dry cacao per 1000 trees, as compared with the average of about  $5\frac{1}{2}$  bags for the whole of Trinidad. The soil is derived from glauconitic calcareous sandstone, closely resembling Greensand in mineralogical composition. Its contents of nitrogen, available phosphate and available potash are extremely high. The parent rock weathers to a bright red-brown incoherent sand, and the derived soil is a deep clay loam, coloured dark chocolate brown by organic matter.

(2) *Yellow calcareous loam soil* (one profile). Esperanza Estate, Montserrat District, Trinidad. Rainfall, 100 in. This soil type is very variable, and generally much less productive of cacao than the last. Yields for the field sampled are around 8 bags per 1000 trees. The soil is derived from a dark blue-grey silt-stone, sometimes highly gypseous (Brasso clay). Its contents of available phosphate and potash are fairly high in the example studied. The parent rock weathers to an ochre-yellow stiff clay, coloured greenish black by organic matter in the shallow surface layer down to 6 in. depth. The profile sampled represents a relatively highly fertile sandy facies of the Brasso clay soil type.

(3) *Brown non-calcareous sandy loam soil* (two profiles). San Pablo Estate and Philippine Estate, Montserrat District, Trinidad. Rainfall, 85 in. This soil type exhibits variable fertility. The first example is much more productive of cacao than the second (yields, 15 bags and 6 bags per 1000 trees respectively). The soil is derived from non-calcareous, semi-coherent, greenish grey, soft sandstone (Brasso sand), sometimes containing potash-bearing glauconite, but usually deficient in phosphate. It is a pale-brown or tawny, friable, porous sandy soil, containing variable amounts of organic matter, the more fertile soils usually being richer in this component, which may exhibit deep penetration.

Data for soil samples taken from the five additional profiles are presented in Table IV.

Table IV. *Comparison of five tropical cacao soil profiles of known productivity*

Depth in.	Index of texture	C/N	Organic matter %	pH	N %	Hydrolysable protein	Celluloses	Re-sistant protein	Lignin humus
(1) Chocolate clay-loam									
IV. San Salvador Estate. Yield, 16 bags cacao per 1000 trees									
0-1½	42	11.3	5.68	6.5	0.29	29.6	15.2	21.0	34.2
1½-4½	54	12.3	11.55	6.9	0.54	22.6	11.6	15.2	50.6
4½-8	50	8.7	5.15	7.0	0.34	34.5	10.9	22.6	31.9
8-12	43	5.5	2.03	7.6	0.21	31.4	9.5	25.8	33.4
V. San Pablo Estate. Yield, 15 bags cacao per 1000 trees									
0-1½	42	8.4	5.01	7.1	0.34	24.2	9.6	18.8	47.4
1½-3	46	10.5	5.71	6.8	0.32	30.8	14.8	25.0	29.4
3-6	43	9.5	5.13	6.7	0.27	26.0	19.6	22.7	31.6
6-12	39	8.7	2.56	6.6	0.17	19.5	23.9	28.9	27.8
12-18	32	9.6	1.56	6.6	0.09	9.5	14.2	25.2	51.1
18-24	36	10.1	1.69	6.5	0.10	13.5	12.1	27.2	47.1
(2) Yellow (Brasso) clay-loam									
VI. Esperanza Estate. Yield, 8 bags cacao per 1000 trees									
0-1½	37	12.5	7.29	7.4	0.34	19.2	19.6	14.6	46.5
1½-3	27	11.1	3.93	7.4	0.21	30.9	25.0	21.0	23.0
3-6	28	8.7	2.09	7.1	0.14	20.3	50.8	20.5	8.5
6-12	29	6.8	1.24	7.2	0.11	41.7	Trace	52.5	5.8
(3) Brown (Brasso) sand									
VII. San Pablo Estate. "Good" area. Yield, 15 bags cacao per 1000 trees									
0-1½	26	10.0	6.33	7.1	0.37	25.5	12.7	19.9	41.9
1½-3	22	8.5	3.95	6.9	0.27	29.0	15.9	18.6	36.5
3-6	16	7.6	2.22	6.7	0.17	27.2	18.4	5.8	48.6
6-12	14	6.8	1.04	6.3	0.09	22.8	22.5	3.2	51.5
VIII. Philippine Estate. "Bad" area. Yield, 6 bags cacao per 1000 trees									
0-1½	25	8.4	4.14	6.1	0.28	26.4	16.7	3.4	53.4
1½-3	22	7.6	2.80	5.7	0.21	32.2	14.9	4.9	48.0
3-6	23	6.1	1.57	5.4	0.15	29.6	25.4	4.7	40.2
6-12	27	3.7	0.48	5.3	0.08	30.0	31.5	8.1	30.4

(1) *Chocolate calcareous clay-loam soil.* The most characteristic feature of these two soil profiles is the regular distribution of each of the various organic fractions in the successive soil layers down to the full depths of the profiles (1 or 2 ft.). Their organic matter component appears to be actively decomposing, as indicated by the uniform content of celluloses throughout. This feature distinguishes the soil type from the black calcareous soil and the two red soils previously considered. The contents of resistant protein and lignin humus are also large in amount, indicating that, although considerable decomposition has already taken place, the humified products have not been removed to any appreciable extent.

(2) *Yellow calcareous (Brasso) loam soil.* The chief feature of this soil profile is the rapid rise in content of celluloses in the three upper shallow

layers down to the 6 in. depth, indicating a downward leaching of carbohydrate residues rendered possible by the open structure of the soil. The rise in cellulose content is accompanied by a corresponding decrease in lignin humus, and a considerable increase in resistant protein.

Below the 6 in. depth, a sudden change in soil composition occurs in this profile. The total organic matter content diminishes greatly in the 6-12 in. layer, and is accompanied by a significant drop in C/N ratio. The sudden change in the C/N ratio is explained by the fact that the chief organic components contained in this subsoil layer are nitrogenous, of which about one-half is easily hydrolysable. The low content of lignin humus in the lower layers is probably accounted for by the lack of cellulosic residues, which has permitted the decomposition of lignin humus to proceed.

The site of the profile is subject to intermittent dry season leaf-fall, which liberates an abundant supply of cellulosic and lignin compounds during these periods. Carbohydrate substances are apparently leached rapidly downwards during the following wet seasons, so that lignin humus is left to decompose in the surface layer. The shallowness of the organic profile affords evidence that the fluctuating supply of plant residues is never very great, though decomposition is rapid, as indicated by the high contents of resistant protein, especially in the subsoil layer. These results may thus be explained by the open structure of the soil, the paucity and inconstancy in the supply of plant residues, and the rapidity with which organic transformations proceed under the prevailing hot humid conditions.

(3) *Brown non-calcareous (Brasso) sandy loam soil.* The two sandy soil profiles show significant differences in total organic matter content and C/N ratio. The more productive soil exhibits the higher values, and is less acid. Its content of resistant protein is much greater in the surface 3 in. layer, but this difference is not shown by the corresponding lower layers. In each profile, as in the last example, cellulosic residues increase considerably downwards, indicating pronounced leaching and penetration. The less productive soil shows a regular downward decrease in lignin-humus content, which is not exhibited by the other profile.

From these observations, it is conjectured that the more productive sandy soil (San Pablo profile) receives a more constant supply of plant residues from regular heavy leaf-falls, with the result that cellulosic substances are always present in sufficient quantity to balance the decomposition of lignin compounds. On the other hand, the less productive soil (Philippine profile) apparently receives an intermittent

supply of plant residues, which is never sufficiently large to maintain a regular supply of cellulosic material.

These differences are in accordance with field observations. A very striking feature of all good cacao fields in Trinidad is the constant presence of a thick layer of loose leaf litter, whereas poor cacao fields, apart from periodic leaf-fall which occurs at the onset of the dry season, usually contain only a very thin irregular layer of litter, exposing numerous bare patches of soil.

*Discussion.* The data obtained by these fractionation methods applied to eight tropical soil profiles indicate the following general trends:

(a) In highly organic surface soil layers, there seems to be no great difference in chemical composition of the organic matter, unless the rate of decomposition greatly exceeds the rate of supply of plant residues.

(b) Under normal circumstances in the hot humid tropics, the cellulosic substances of fresh plant residues appear to be readily decomposed and to disappear first. At the same time, the lignin humus is probably also decomposed, but not so rapidly. If the supply of plant material is inadequate, the content of cellulosic substances soon falls to a low level. Furthermore, the amount of available lignin compounds is insufficient to balance their decomposition, and the lignin-humus content consequently diminishes. Thus, a low cellulose content, accompanied by a low lignin-humus content, may be regarded as indicating an inherently infertile or an exhausted or deteriorated soil (for example, the Stony Hill Jamaican red soil, Table III).

(c) The content of resistant protein appears to be connected with soil fertility. When the supply of plant residues is plentiful, but the soil lacks downward drainage (heavy and medium-textured soils), the content of resistant protein apparently increases with increasing depth of soil. The same result may occur in a poor soil inadequately supplied with cellulosic substances, in which microbiological activity has consequently fallen to a low level, and in which downward leaching has not proceeded far enough to affect the distribution of nitrogenous compounds. The addition of organic matter to such a soil would probably quickly improve its fertility. An exceptionally low content of resistant protein may be regarded, however, as indicative of profound deterioration, so that improvement in fertility, following the application of fresh plant residues, in this case may be very slow.

(d) (i) Below the highly organic surface layer the same amount of plant material added to the soil may exert varying effects, depending mainly on the degree of permeability of the soil. Thus, in heavy imper-

vious clay soils, the greater part of the organic transformations occurs only in the shallow surface layer within which aeration is adequate. The content of cellulose residues may diminish rapidly down the profile, even in a "good" clay soil, such as the Trinidadian black calcareous clay. In the lowermost layers, organic matter occurs mainly as nitrogenous substances which have been slowly leached downwards from the decomposing surface organic layer. The controlling factor seems to be the depth of the permeable, well-aerated surface layer of soil. It therefore follows that any agricultural practice which depletes a heavy clay soil of its surface organic matter will diminish aeration and thus retard the normal processes of decomposition. Since percolation is greatly restricted in a clay soil, the downward transportation of the products of decomposition may not affect the composition of the lower layers for some time, and soil exhaustion may thus proceed only slowly.

(ii) In highly permeable sandy soils, the decomposition processes are extremely rapid, since aeration is adequate even to great depths. Unrestricted downward percolation also favours the uniform distribution of organic matter throughout the profile, and leaching may be so intense that much of the plant material is carried downwards before it has completely decomposed. The decomposition of organic matter may thus be very rapid in such soils, and a heavy leaf-fall is required to maintain soil fertility. In the lowermost layers, cellulosic substances may be deficient, and even low lignin-humus contents may occur. In such cases, increased supplies of organic materials would cause quick recovery, but agricultural exploitation would bring about rapid deterioration.

(iii) Evidently, therefore, the most suitable soils for humid tropical agriculture are loams and silts, in which the percolation of water is neither too slow nor too rapid. They deteriorate at a relatively slow rate, and recover correspondingly quickly. Where methods of "shifting cultivation" can be confined to specific areas of land, it would be preferable to choose the intermediate physical types of soil, and to maintain clays and sands under perennial crops. When forest or bushland is cleared prior to planting a perennial crop, the most important consideration should be the establishment of a thick ground cover in order to restrict the loss of natural organic matter.

#### (E) *Comparison of temperate and tropical peats*

Waksman & Stevens have made extensive studies of the chemical and microbiological relationships of peat, but as far as the writer is aware, no investigations of tropical organic accumulations have yet been



attempted. Accordingly, two types of tropical peat occurring in the north-west district of British Guiana, South America (locally known as black and red pegasse), were examined by the simplified fractionation method. For purposes of comparison, two samples of British moorland peat and one of fen peat were also examined.

The data obtained for the British and the South American (British Guiana) peats are presented in Table V, and data for highmoor and low-moor peats examined by Waksman & Stevens<sup>(11,12)</sup>, are presented in Table VI, the necessary recalculations having been made in order to bring their results to a comparable basis of recoverable organic matter.

Table V. *Comparison of British and South American (British Guiana) peats*

	C/N	pH	Benzol- alcohol	Hydro- lysable protein	Cellu- loses	Resistant protein	Lignin humus
(A) British peats							
Yorkshire moorland peat	29.8	3.7	9.8	7.8	24.8	3.9	53.7
Lewis (Scotland) peat	27.0	4.1	6.8	7.6	34.8	3.9	46.9
Cambridgeshire fen peat	15.3	6.9	1.6	12.2	14.2	12.8	59.2
(B) British Guiana peats							
Black pegasse	15.9	4.8	7.8	12.6	24.0	7.0	48.7
Black pegasse	17.0	5.0	7.6	10.0	25.8	6.4	50.2
Red pegasse	26.7	3.7	12.7	6.9	10.6	5.9	64.0
Red pegasse	31.8	3.7	13.3	5.8	12.6	4.0	64.5

Table VI. *Peats examined by Waksman & Stevens*

	Ether extract	Total celluloses	Total proteins	Lignins
Highmoor peat, Maine, U.S.A.	4.8	55.3	5.2	34.7
Highmoor peat, Germany	3.9	46.2	6.6	43.3
Lowmoor peat, New Jersey, U.S.A.	0.9	14.4	31.3	53.4
Lowmoor peat, Florida, U.S.A.	3.0	16.4	26.0	54.6

(A) *Temperate peats.* The Yorkshire and the Lewis peats, which produce infertile soils, contain large amounts of benzol-alcohol extractable matter and small amounts of resistant protein, which may be considered to indicate incomplete organic decomposition. Nevertheless, they both contain large amounts of cellulosic substances, indicating that thorough draining and liming should promote rapid breakdown of organic matter through increased aeration and lessened acidity, so that the materials might become more favourable for the growth of crops. Recent work at the Macaulay Institute for Soil Research, Aberdeen, has indeed demonstrated that draining and liming brings about a remarkable improvement in the fertility of Lewis peat.

The Cambridgeshire fen peat, on the other hand, contains only a

small amount of benzol-alcohol extractives, a medium amount of cellulosic substances, and a large amount of resistant protein. The high fertility of the fenlands is well known, and it is evident from the analytical figures that active decomposition of the organic matter component is largely responsible for this feature. Although the fenlands have been under cultivation for many generations, their soil organic matter still exhibits a well-balanced composition. Evidently, a highly organic soil does not necessarily deteriorate rapidly in a temperate climate.

The highmoor and the lowmoor peats examined by Waksman & Stevens show similar differences to those which obtain between the Yorkshire and Lewis peats and the Cambridgeshire fen peat. The highmoor peat contains the greater amounts of ether extractives and cellulosic substances, and a much smaller amount of protein; it is generally less fertile than lowmoor peat.

(B) *Tropical peats*. Pegasse in British Guiana occurs in brackish swamplands bordering tidal rivers and creeks. The river banks are lined with mangrove, immediately behind which occurs an association of palms (*Manicaria saccifera* and *Euterpe* spp.) with dicotyledonous trees (*Simphonia globulifera*, *Tabebuia* sp. and *Pterocarpus draco*). The residues of this association produce black pegasse. Farther away from the river banks, where conditions are presumably less brackish, the greater part of the swamplands supports a low vegetation, in which *Simphonia* and *Tabebuia* occur, together with species of low-growing trees and abundant swamp lily. This vegetation produces red pegasse.

Black pegasse yields much more fertile soil when drained and tilled than red pegasse. Follett-Smith, in his report on the soils of the north-west district of British Guiana<sup>(13)</sup>, states that the aboriginal Indians account for the differences in the fertility of black and red pegasse on the supposition that the black swamplands are better drained, whilst the water of the red pegasse swamps is more stagnant. Auger borings failed, however, to confirm this belief.

The results (Table V) show that the black pegasse has a lower C/N ratio, and contains significantly more cellulosic substances and hydrolysable and resistant nitrogenous substances than the red pegasse, though it contains less extractives and lignin humus. These results suggest that the red pegasse would break down only very slowly on aeration, because the inadequate supply of celluloses and the high C/N ratio would limit micro-organic activity and induce nitrogen starvation. The high lignin-humus content of the red pegasse indicates that the original plant material was somewhat woody in nature, and therefore likely to be very resistant to decay. On the other hand, black pegasse,

having a much lower degree of acidity, and containing greater amounts of cellulosic substances, would be more prone to attack by micro-organisms, so that drainage and tillage alone would be expected to cause rapid decomposition, resulting in the formation of a fertile soil.

On the whole, black pegasse somewhat resembles the British examples of peat, and the temperate highmoor peats examined by Waksman & Stevens (high cellulose, relatively low total protein contents), whilst red pegasse contains more lignin humus, and is much less decomposed. In order to reclaim red pegasse land for agriculture, thorough draining and heavy liming would be required, as well as the addition of nitrogenous manures, whilst black pegasse land would only need draining in order to render it productive of crops. Where black pegasse occurs within the sugar-cane lands of British Guiana, it gives rise to fertile soil when drained and tilled.

### III. SUMMARY

1. Waksman's simplified scheme of fractionation was used in attempts to trace the organic transformations occurring in some tropical samples of composts, soils and peats.

2. It was found that a useful partition of the nitrogenous substances into hydrolysable and resistant fractions could be effected by boiling with 5 per cent sulphuric acid for 6 hours.

3. Two profile layers of a black calcareous Trinidadian clay soil were compared with two horizons of a prairie soil examined by Waksman & Stevens. The surface layers of both soils were found to be similar in chemical characters, but the lower subsoil layers of the Trinidadian soil gave evidence of a more advanced and profound degree of organic decomposition.

4. Serial soil samples, taken from eight undisturbed natural profiles representative of humid tropical soil types occurring in Jamaica and Trinidad, were similarly examined.

(a) With the exception of two soils known to be much less fertile, the surface layers showed close similarity in chemical composition.

(b) The lower organic layers (about 12 in.) were found to contain insignificant amounts of celluloses, but large amounts of hydrolysable and resistant nitrogenous substances, signifying an advanced stage of decomposition.

(c) Successive subsoil layers showed diminishing C/N ratios ranging down to 5 or 4. Apparently nitrogenous complexes and lignin humus are much less stable under hot humid tropical conditions than under temperate conditions.

(d) A rapid decrease in resistant protein appears to indicate soil deterioration and exhaustion, even though the amount of hydrolysable protein may still be large.

(e) Lignin humus apparently decreases only when the celluloses have first been decomposed and removed.

(f) The decomposition of plant residues applied to the soil surface is affected by soil structure and texture. In the case of impervious clay soils, the rate of deterioration of the added organic matter appears to be relatively slow, but recovery may also be slow. In the case of open sandy soils, deterioration is very rapid, but recovery may also be rapid. Loam and silt soils appear to be the most suitable types for utilization by "shifting cultivation".

5. Samples of two kinds of tropical peat (pegasse) occurring in British Guiana were compared with British peats, and with results published by Waksman & Stevens for highmoor and lowmoor peats. The evidence obtained revealed the main differences between the tropical peats with regard to degree of decomposition, and indicated the most likely means of converting them into fertile soils.

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# CHANGES IN THE COMPOSITION OF GUANO DURING STORAGE

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(With Two Text-figures)

THE term "guano" is applied almost wholly to the excrement of sea-birds and, since it contains a high proportion of nitrogen and phosphorus, guano forms a very valuable fertilizing material. The Union of South Africa has a large number of small islands scattered round the coast which are used as breeding grounds by various sea-birds. The deposits of guano from different islands vary considerably and are therefore systematically mixed to obtain an approximately uniform grade of material. The average analytical results for the years 1921-8 were: 10.0 per cent nitrogen, 11.2 per cent phosphoric oxide, 2.0 per cent potash.

As a guano deposit becomes older, it tends to lose nitrogen and water-soluble compounds and becomes "phosphatic". The relationships between the various components of a "normal" guano are shown (Table I) by a series of correlation coefficients worked out from the data obtained under the Fertilizer Act during the years 1921-8.

Table I

	Water-soluble phosphoric oxide	Potash	Lime	Nitrogen
Total phosphoric oxide	-0.34	-0.18	+0.85	-0.46
Water-soluble phosphoric oxide	—	+0.44	-0.59	-0.70
Potash	—	—	-0.22	+0.50
Lime	—	—	—	-0.55

The limit of  $r$  for  $P < 0.01$  is  $\pm 0.33$ .

It is clear that these factors can be divided into two classes, namely, (a) total phosphoric oxide and lime, and (b) water-soluble phosphoric oxide, potash and nitrogen. Moisture and rain facilitate the removal of soluble compounds and allow the formation of insoluble compounds. In

addition to these direct effects the nitrogen compounds are converted into ammonium compounds which are not only soluble but are also volatilized when, at a later stage, the guano dries out. There is thus an additional loss of valuable fertilizing material. In arid tropical climates the rapid desiccation of the deposits inhibits the conversion of the complex nitrogen compounds to ammonium salts.

#### EXPOSURE TO DRY AND MOIST ATMOSPHERES

Six samples of guano of South African origin were stored under two different atmospheric conditions, namely, (a) the samples were allowed to become "air-dry", and (b) the samples were kept in an atmosphere saturated with water vapour. After a period of about 7–10 weeks in each case the samples were again analysed and the results compared with the data for the original samples. In addition to the usual determinations, the uric acid content of the samples was determined by Woodman's method for fowl excreta (5).

The average values for these six guanos were: moisture, 13.84 per cent; ash, 45.20 per cent; silica, 13.13 per cent; total phosphoric oxide, 12.16 per cent; lime, 11.97 per cent; potash, 2.13 per cent; nitrogen, 10.23 per cent. As a measure of the amounts of material which might be removed by leaching, the following were the average results for water-soluble components: phosphoric oxide, 3.26 per cent; potash, 1.66 per cent; nitrogen, 3.10 per cent; ammonia-nitrogen, 2.46 per cent. It is interesting to note that about 80 per cent of the soluble nitrogen is in the form of ammonia, while only about 80 per cent of the total potash is immediately soluble. The amount of silica varied widely, the results varying from 5.8 to 24.7 per cent, and this can only be accounted for on the assumption that the silica is an adventitious factor derived from the sand mechanically mixed with the samples during their collection. The appearance of the insoluble residue strongly supports this view. When the results are expressed on the moisture- and silica-free basis the figures obtained are so closely comparable with those for the best Peruvian guano that it is clear that there is no inherent difference in the guanos from the two sources.

In determining the moisture content of the samples in each series, corrections were made to the total loss of weight at 105° C. for the amounts of carbon dioxide and ammonia driven off at this temperature. The average moisture content in each series is given in Table II. Under similar conditions of storage the samples all tended to absorb the same amount of moisture; an air-dry sample has about 8 per cent of moisture,

## 180 *Changes in the Composition of Guano during Storage*

while, in a moist atmosphere, the samples absorb moisture up to about 25 per cent. The amount of ammonia evolved during the drying at 105° C., calculated as nitrogen, may be termed "volatile" nitrogen. The ratio of "volatile" to total nitrogen may be used to eliminate the effects of varying moisture. The average figures obtained from these six samples are given in Table II.

Table II

Series	Moisture	"Volatile" nitrogen	Ratio × 100
Original	13.84	0.79	7.7
Dry	8.04	0.37	3.5
Moist	23.32	0.77	9.4

It is clear that the proportion of "volatile" nitrogen tended to increase with the moisture content. There is a high degree of correlation between the ratio and the moisture content, namely,  $r = +0.81$ , but the regression lines are curved, as shown in Fig. 1, where the average values of the ratio are plotted against each successive 2.5 per cent increase in the moisture content. The ratio tends to reach a maximum of about 10 per cent as the limiting value, so that there appears to be a limit to the amount of "volatile" nitrogen which can be retained by the moist sample.

In Table III the average results for each series are reported on the moisture-free basis.

Table III

### *Non-nitrogenous components*

Series	Ash	Silica	Total phosphoric oxide	Lime	Potash
Original	52.70	15.63	14.13	13.90	2.47
Dry	52.52	15.56	14.14	13.95	2.38
Moist	53.17	15.65	14.38	14.06	2.47

### *Nitrogenous components*

Series	Total nitrogen	Uric acid nitrogen	Water-soluble nitrogen	Ammonia nitrogen
Original	11.92	4.77	3.61	2.85
Dry	11.56	4.70	3.14	2.44
Moist	10.98	3.64	3.96	3.07

It will be seen that the conditions of storage directly affect the nitrogen content of the guano, the effects being most evident in the samples stored in a moist atmosphere. Any small changes in the other components may be ascribed to the changes in the relative amounts of the nitrogen content.

The significance of the various differences in the nitrogen contents was tested by the *t* method of Fisher<sup>(1)</sup>, the results being given in Table IV.

Table IV

Nitrogen	Dry original	<i>t</i>	Moist original	<i>t</i>
Total	-0.36	3.83	-0.94	1.43
Uric acid	-0.07	1.25	-1.13	2.37
Water-soluble	-0.47	3.61	+0.35	4.54
Ammonia	-0.41	3.83	+0.22	4.23

These results indicate the changes in distribution of the nitrogen under different conditions of storage. When the samples became "air-dry" there was a significant loss of 0.36 per cent of nitrogen. When stored in a moist atmosphere there was an average loss of 0.94 per cent but this does not appear to be significant, the source of the large difference being mainly confined to two samples which were exposed for a longer time to the moist atmosphere.

In the dry condition there was no change in the uric acid content, but under moist conditions the "uric acid" nitrogen decreased by 1.13 per cent. During air-drying the loss of "soluble" nitrogen is due to the volatilization of ammonia. In the moist samples the "soluble" nitrogen increased significantly by 0.35 per cent of which 0.22 per cent is accounted for by "ammonia" nitrogen. It is clear that, in the presence of moisture, the uric acid is converted into ammonium compounds which are, at least partly, retained by the moisture present in the guano.

#### EFFECT OF ALTERNATE WETTING AND DRYING

In practice the guano will probably be subjected to alternate periods of wetting and drying. The ammonium compounds in the first instance will tend to be volatilized during the second phase. The loss of nitrogen will thus be intensified apart from losses due to possible leaching. This point was studied by moistening the guanos with water to form a thick paste. The samples were allowed to become "air-dry" and, after analysis, this process was repeated. For comparison, samples of the guanos were exposed for 6-7 weeks to become air-dry in the normal way. The average moisture content of the original samples was 14.0 per cent, while the average moisture content of the "air-dry" guanos was 7.8 per cent, irrespective of the treatment received. The previous figure obtained was 8.04 per cent, and it is apparent that the moisture content of an "air-dry" guano lies within narrow limits. In view of these results it is suggested that, in practice, analytical results should be standardized for guanos on this basis.



## 182 *Changes in the Composition of Guano during Storage*

The average results obtained for the guanos after the different treatments are given in Table V.

Table V

Treatment	Exposure in days	Phosphoric oxide	Lime	Total nitrogen	Water- soluble nitrogen	Ammonia nitrogen
Original	—	13.60	14.98	10.96	3.40	2.66
First wetting	16	13.86	15.12	10.37	2.79	2.11
Second wetting	15	14.03	15.40	9.93	2.49	1.78
Air-dried	44	—	—	10.53	2.98	2.43

It will be seen that there is a definite loss of nitrogen in all cases during storage, so that there is a general deterioration in value. It is clear, however, that this change is more rapid when the guano has been moistened. The losses are more evident when the difference between the nitrogen content, "as received" and that after treatment are studied. These average differences of nitrogen content are given in Table VI.

Table VI

	First wetting	Second wetting	Air-dry
Total nitrogen	0.59	1.03	0.43
Water-soluble nitrogen	0.61	0.91	0.42
Ammonia nitrogen	0.55	0.88	0.43

In these cases all the samples finally became "air-dry" and no ammonia or soluble nitrogen was fixed by the moist guano. In every case there was a loss of nitrogen. It will be seen that the loss is due practically to loss of ammonia, so that, when the samples dry out after wetting, the ammonia formed by decomposition of the uric acid tends to be volatilized. The amount of nitrogen lost in two treatments with water is at least double the amount lost when the guano is allowed to become "air-dry" without wetting for a much longer period of time. It must also be remembered that the "air-dry" samples initially contained moisture in excess of that present in the "air-dry" state, so that initially conditions for decomposition were fairly favourable.

The effects of the different treatments are more apparent when the average weekly rates of loss are compared as shown in Table VII.

Table VII. *Average weekly rates of loss*

	First wetting	Second wetting	Air-dry
Total nitrogen	0.26	0.21	0.07
Water-soluble nitrogen	0.27	0.14	0.07
Ammonia nitrogen	0.25	0.15	0.07

These figures indicate clearly the more rapid loss of nitrogen from the moistened samples. In the case of the first wetting and also in the "air-dry" samples the rates of loss of the three classes of nitrogen are the same, but in the second wetting the rate of loss of the total nitrogen was greater than in the case of the soluble or ammonia nitrogen. Since the loss of soluble nitrogen could be accounted for by the loss of ammonia it seems evident that some process of denitrification is taking place. All the conditions for such a process are present in a thoroughly wetted guano.

From the above results it is clear that two main sources of change are present in guanos, namely, (a) decomposition, with the formation of soluble nitrogen compounds, and (b) leaching, with the loss of soluble salts. The importance of keeping guano in as dry a state as possible is therefore clear.

#### DECOMPOSITION OF THE URIC ACID IN GUANO

It has been shown that the decomposition of uric acid into ammonium salts takes place most readily when the guano is moist. A series of experiments was carried out in which the guano was allowed to remain in contact with water, and the changes in the ammonium content of the solution were determined.

Six parallel sets of solutions were prepared according to the following scheme:

A. A solution of known volume, generally 500 ml., was prepared by shaking up the required weight of guano with water and filtering into a stoppered flask.

B. A set of solutions was prepared with the guano remaining in contact with the solution. Before determining the ammonia the solutions were filtered in each case.

C. A set of solutions, similar to B, was prepared but each contained a crystal of thymol and a few drops of chloroform.

D. A solution was prepared, as in A, but contained 10 ml. of hydrochloric acid per 100 ml.

E. A set of solutions was prepared, similar to B, but each solution contained hydrochloric acid in the same concentration as in D.

F. A set of solutions was prepared, similar to E, but each solution contained thymol and chloroform.

In all cases 2 g. of guano were used for each 100 ml. of water and the flasks were tightly stoppered to prevent loss. The flasks were shaken at intervals and the solutions filtered before determining the ammonia.

## 184 *Changes in the Composition of Guano during Storage*

A typical set of results is given in Table VIII, in which the ammonia is expressed in terms of nitrogen per 100 g. of guano.

Table VIII

Time in days	A	B	C	D	E	F	B - A
0	3.08	—	—	4.49	—	—	—
1	3.14	3.22	—	4.50	4.43	4.29	0.14
2	3.18	6.10	3.10	4.51	4.39	4.29	3.02
3	—	7.39	—	—	—	—	4.31
4	3.20	8.50	—	—	—	—	5.42
5	—	8.35	3.08	—	4.32	4.40	5.27
6	—	8.59	—	4.51	4.33	4.30	5.51
7	3.23	8.62	—	—	—	—	5.54
8	—	8.63	3.09	—	4.30	4.28	5.55
9	3.20	8.62	—	4.45	—	—	5.54
10	—	—	—	—	—	—	—
11	—	8.50	—	—	—	—	5.42
12	3.25	—	—	4.47	4.35	4.38	—
Average			3.09	4.49	4.35	4.32	

The only case where there was a progressive increase in the ammonia content with time was in B, where the guano remained in contact with its untreated aqueous solution. The total nitrogen content of this particular sample of guano was 10.0 per cent, so that 86 per cent of the nitrogen was converted into ammonia. The progressive increase of ammonia must be due to the decomposition of insoluble nitrogen compounds, since filtered solutions, as A, do not show this increase. These insoluble compounds are uric acid and its salts, and since the changes are inhibited by antiseptics, as in C, bacterial decomposition is indicated. Truszkowski (4) has shown that uricolysis by kidney extracts must be mainly due to bacterial action and that growth of the bacteria does not occur in solutions below pH 6.0. He concludes that the active uricolytic agent is of the nature of a contact catalyst associated with cell fragments in suspension. The results of series C and D are in complete accordance with these views.

The increase of ammonia ceases about the 7th-8th day. In the solution A there was an increase of "ammonia" nitrogen of about 0.15 per cent of the guano, which can be accounted for by the uric acid dissolved in the filtered solution. The amount of uric acid in the aqueous solution averaged 0.0090 g. per 100 ml., which is in excellent agreement with the value of 0.00897 g. per 100 g. for the solubility of uric acid (2).

There is also an initial difference of 1.41 per cent in the nitrogen content of the two control solutions A and D. This must be due to the interaction of insoluble ammonium urate with the hydrochloric acid to give soluble ammonium chloride. The change of ammonia nitrogen from

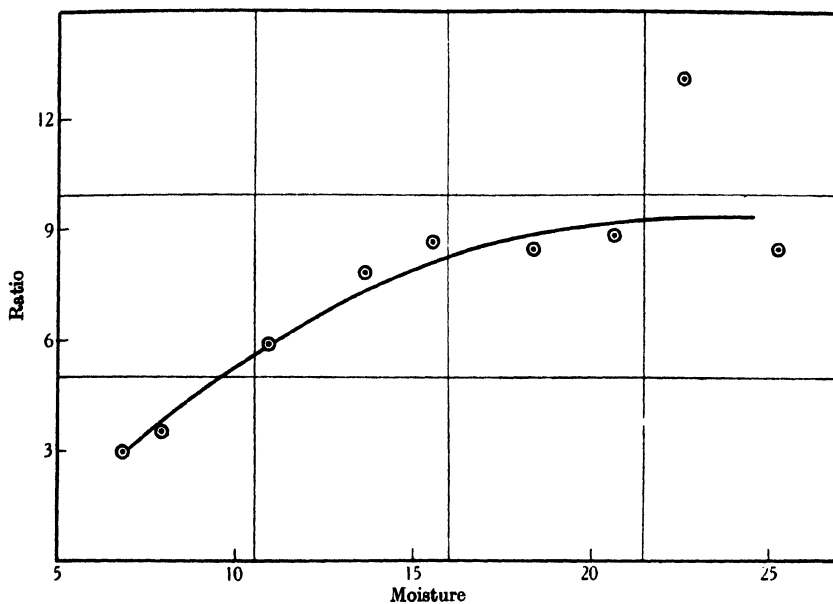


Fig. 1

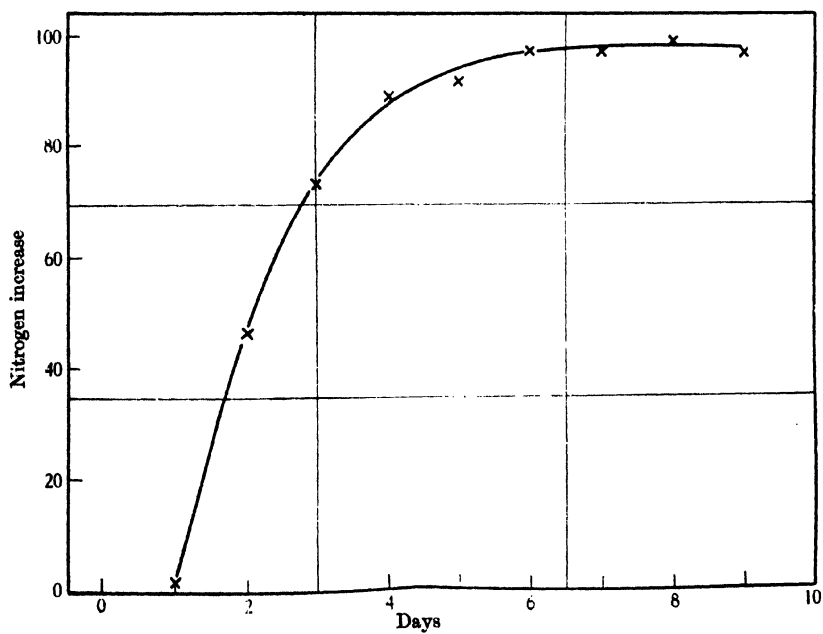


Fig. 2

## 186 *Changes in the Composition of Guano during Storage*

4.49 to 8.63 per cent must be due solely to the decomposition of uric acid. The difference of 4.14 per cent nitrogen is equivalent to 12.4 per cent of uric acid in the guano. The amount actually found by Woodman's method (5) was 12.2 per cent.

When the data for the increase in "ammonia" nitrogen content of the solution were plotted against time a logarithmic curve of the type of a monomolecular reaction was obtained. There was a short period of induction, in agreement with the observation of Truszkowski that the uric acid was decomposed only after the bacteria had commenced to grow. The average values for four different samples of guano, expressed as percentages of the final theoretical amounts of "ammonia" nitrogen produced by the decomposition, are shown in Fig. 2.

A further experiment was carried out to determine whether a filtered extract of guano contains a definite uricolytic agent. Three 100 ml. flasks, containing 0.164 g. of uric acid, were filled to the mark with a filtered guano extract, well stoppered and shaken at intervals. During the period of standing the white deposit of uric acid gradually disappeared. The results for the ammonia content of the solutions in terms of ml. of *N*/10 alkali per 25 ml. of solution are given in Table IX.

Table IX

Time in days	Control	Control + uric acid
0	11.05	—
4	11.48	20.80
5	—	20.90
11	11.50	21.12

After 11 days the difference between the two solutions was equivalent to 0.162 g. of uric acid, so that 98.8 per cent of the uric acid had been converted into ammonium salts. In this connexion Shimoda (3) states that uric acid is completely destroyed by *N*/10 sodium hydroxide. To ascertain whether the changes described above might not be due to the alkaline nature of the guano extract a series of experiments was carried out with a guano extract to which thymol and chloroform had been added. The results are given in Table X in terms of ml. of *N*/10 alkali per 25 ml. of solution.

Table X

Time in days	Uric acid + <i>N</i> /10 sodium hydroxide	Control	Uric acid + extract + antiseptic
7	0.33	10.98	—
9	0.24	—	8.92
14	0.29	—	—
15	—	11.25	8.93

It is clear that, in the presence of  $N/10$  alkali, there has been no production of ammonium salts. There can be no doubt that in guano there is definitely a uricolytic agent which is bacterial in nature.

It is interesting to note that the amount of ammonia in the series with the uric acid is less than in the control solution. This is readily explained by the fact that the uric acid reacted with the ammonium salts normally present in the guano extracts to form an insoluble ammonium urate, which was removed when the solutions were filtered before the determination of the ammonia.

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# A NOTE ON THE METHOD OF "DIFFERENTIAL REGRESSION" IN ANALYSIS OF VARIANCE

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SOME time ago, the author (Hendricks, 1935*a*) pointed out a modification which may occasionally be desirable in the application of co-variance analysis to experimental data. It was suggested that, in some instances, it is more logical to base the analysis on the assumption that the slope of the line of regression varies from class to class than on the assumption that the regression coefficient has the same value for each class of observations.

In the method of analysis proposed, no correction was provided to make proper allowance for sampling errors in the regression coefficients involved. The author's only defence of this oversight lies in the fact that the necessity for making such a correction, even in the case of the conventional method of analysis proposed by Fisher (1932), was not generally recognized at that time. It is now quite apparent that such a correction is of greater importance when the analysis is based on the method of "differential regression" than when a single, average regression coefficient is used, as in the case of the conventional co-variance analysis, for the sampling errors of the individual regression coefficients are always greater than those of the average regression coefficient.

The appropriate correction to be applied in the conventional analysis, employing a single regression coefficient, has been discussed by Bartlett (1934), Fisher (1934), and Yates (1934). Although it may be possible to deduce the appropriate correction for the case of the method of "differential regression" in the same manner, the present author has arrived at a satisfactory solution of the problem by a slight extension of some principles discussed in a recent paper (Hendricks, 1935*b*).

For illustrative purposes, it will be sufficient to consider a simple application of co-variance analysis. The extension of the method to more complex problems involves no difficulty.

Suppose we have a set of measurements in each of  $k$  classes. Let  $y_{ij}$  represent the  $j$ th measurement in the  $i$ th class. We wish to determine

whether or not the variance "between classes",  $S'^2$ , is significantly greater than the variance "within classes",  $S^2$ , after the measurements have been adjusted, by the method of "differential regression", for the effects of a correlated variate,  $x_{ij}$ .

The variance "within classes" is given correctly by the procedure outlined in the author's earlier paper (Hendricks, 1935*a*). However, to find the variance "between classes" in the light of the newer theory, we proceed as follows.

Let  $\bar{y}_i$  represent the mean of the  $y_{ij}$  in the  $i$ th class. Let  $\bar{Y}_i$  represent the adjusted value of that mean, defined by the relation

$$\bar{Y}_i = \bar{y}_i - b_i (\bar{x}_i - \bar{x}), \quad \text{.....(1)}$$

in which  $\bar{x}_i$  represents the mean of the  $x_{ij}$  in the  $i$ th class,  $\bar{x}$  represents the mean of all of the  $x_{ij}$  in the  $k$  classes, and  $b_i$  represents the regression coefficient appropriate to the measurements in the  $i$ th class.

The value of the regression coefficient,  $b_i$ , is given by the relation

$$b_i = \frac{\sum_j [(x_{ij} - \bar{x}_i) y_{ij}]}{\sum_j (x_{ij} - \bar{x}_i)^2}. \quad \text{.....(2)}$$

By the law of propagation of error, we have for the squared standard error of  $\bar{Y}_i$

$$\sigma_{\bar{Y}_i}^2 = \sum_j \left( \frac{\partial \bar{Y}_i}{\partial y_{ij}} \right)^2 \sigma^2, \quad \text{.....(3)}$$

in which  $\sigma^2$  represents the squared standard error of a single measurement.

From a consideration of equations (1) and (2), we may easily deduce the relation

$$\sum_j \left( \frac{\partial \bar{Y}_i}{\partial y_{ij}} \right)^2 = \frac{1}{n_i} + \frac{(\bar{x}_i - \bar{x})^2}{\sum_j (x_{ij} - \bar{x}_i)^2}, \quad \text{.....(4)}$$

in which  $n_i$  represents the number of measurements in the  $i$ th class.

Since the weight of a statistic is inversely proportional to the squared standard error of that statistic, the weight,  $p_i$ , of  $\bar{Y}_i$  is given by the relation

$$\frac{1}{p_i} = \frac{1}{n_i} + \frac{(\bar{x}_i - \bar{x})^2}{\sum_j (x_{ij} - \bar{x}_i)^2}. \quad \text{.....(5)}$$

The variance "between classes",  $S'^2$ , may then be obtained from the relation

$$S'^2 = \frac{1}{k-1} \sum_i p_i (\bar{Y}_i - \bar{Y})^2, \quad \text{.....(6)}$$

in which  $\bar{Y}$  is the weighted mean of the  $\bar{Y}_i$ , defined by

$$\bar{Y} = \frac{\sum_i p_i \bar{Y}_i}{\sum_i p_i}. \quad \text{.....(7)}$$



## 190 "Differential Regression" in Analysis of Variance

The variance "between classes" given by equation (6) may properly be compared with the variance "within classes" by the familiar  $z$  test.

As an application of the above theory to a particular set of data, the author recalculated the analysis of variance of the gains in live weight of fourteen white rats, which was used as an illustration in his earlier paper (Hendricks, 1935*a*). The results are summarized in Table I.

Table I. *Analysis of variance of gains in live weight corrected for differences in feed consumption. (Method of differential regression)*

Source of variability	Degrees of freedom	Sum of squares	Mean square
Between diets	1	51.02	51.02
Within diets	10	1153.62	115.36
Total	11		

The analysis of variance of the same data obtained by the conventional procedure, employing a single regression coefficient, as revised by Fisher (1934), is presented in Table II.

Table II. *Analysis of variance of gains in live weight corrected for differences in feed consumption. (Ordinary variance and co-variance analysis)*

Source of variability	Degrees of freedom	Sum of squares	Mean square
Between diets	1	46.66	46.66
Within diets	11	1158.41	105.31
Total	12		

A comparison of Tables I and II with the corresponding tables in the author's earlier paper clearly demonstrates the importance of making due allowance for sampling errors in the regression coefficients involved in the analysis.

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# SHEEP NUTRITION

## I. MEASUREMENTS OF THE APPETITES OF SHEEP ON TYPICAL WINTER RATIONS, TOGETHER WITH A CRITICAL STUDY OF THE SHEEP-FEEDING STANDARDS

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### INTRODUCTION

THE formulation of feeding standards for use in the rationing of sheep is based on a knowledge of (1) the capacity for food consumption, in terms of lb. dry matter, of sheep at all live-weights up to slaughter; (2) their maintenance requirements at these live-weights and their production requirements, per lb. live-weight increase, at all stages of growth and fattening, both these forms of requirement being expressed in terms of starch equivalent and digestible protein.

Table I. *Summary of Prof. T. B. Wood's feeding standards  
for sheep*

Live-weight lb.	Appetite, dry matter per week lb.	Maintenance requirement (per week)		Total protein equivalent required per week lb.	Production requirement per lb. live-weight increase, lb. starch equivalent
		lb. starch equivalent	lb. digestible protein		
20	8	3	0.08	$\frac{3}{4}$	1
30	11	4	0.12	1	1
40	13	$4\frac{1}{2}$	0.16	1	$1\frac{1}{4}$
50	15	$5\frac{1}{2}$	0.20	$1\frac{1}{4}$	$1\frac{1}{2}$
60	17	$6\frac{1}{2}$	0.24	$1\frac{1}{2}$	$1\frac{1}{2}$
70	19	7	0.28	$1\frac{1}{2}$	$1\frac{1}{2}$
80	21	$7\frac{1}{2}$	0.32	$1\frac{3}{4}$	$1\frac{1}{2}$
90	$22\frac{1}{2}$	$8\frac{1}{2}$	0.35	$1\frac{3}{4}$	$1\frac{3}{4}$
100	24	9	0.38	$1\frac{3}{4}$	2
110	$25\frac{1}{2}$	$9\frac{1}{2}$	0.42	$1\frac{3}{4}$	$2\frac{1}{4}$
120	27	10	0.46	$1\frac{3}{4}$	$2\frac{1}{2}$
130	$28\frac{1}{2}$	$10\frac{1}{2}$	0.50	$1\frac{3}{4}$	$2\frac{3}{4}$
140	30	11	0.54	$1\frac{3}{4}$	3
150	$31\frac{1}{2}$	$11\frac{1}{2}$	0.58	$1\frac{3}{4}$	$3\frac{1}{4}$
160	33	12	0.62	$1\frac{3}{4}$	$3\frac{1}{2}$
170	34	$12\frac{1}{2}$	0.66	$1\frac{3}{4}$	4
180	35	13	0.70	$1\frac{3}{4}$	$4\frac{1}{4}$
190	36	$13\frac{1}{2}$	0.74	$1\frac{3}{4}$	$4\frac{1}{4}$
200	37	14	0.78	$1\frac{3}{4}$	$4\frac{1}{2}$

The only attempt in this country to draw up a complete table of feeding standards for sheep was made by the late Prof. T. B. Wood in 1928(1,2). Since certain aspects of Prof. Wood's proposals have recently been subjected to criticism, and since it is the object of the present inquiry to attempt to decide how far such criticism is justified, it is necessary to record these proposals in full (Table I).

#### SECTION I. APPETITES OF SHEEP AT DIFFERENT LIVE-WEIGHTS

It will be noted from Table I that the appetite of the standard sheep of 100 lb. live-weight is given as about 3.4 lb. of dry matter per day. Prof. J. A. S. Watson *et al.*(3), however, have pointed out that this figure is substantially higher than that given by other authorities. Kellner's figure is 2.6 lb. of dry matter for an animal of similar live-weight at 8-11 months of age, while Henry and Morrison give a slightly higher figure, namely, 2.7-3.1 lb.

From a statistical analysis of the results of a large number of sheep-feeding trials carried out in this country in the past, Prof. Watson *et al.*(3) concluded that, with the ordinary type of British winter ration, the dry-matter consumption per day of sheep is unlikely to exceed 2.6-2.7 lb. per 100 lb. live-weight, and to secure this, from one-third to one-half of the dry matter should be fed as air-dry food. They recognized, however, that the character of the foods composing the ration might influence the amount consumed by sheep and stated that well-balanced rations consisting mainly or entirely of palatable dry foods may result in a rather higher consumption of dry matter, this under favourable circumstances rising to as much as 3 lb. or more per day.

In a further paper(4) the same authors discuss the results of feeding trials designed to measure the appetites of different breeds of sheep. They found that the sheep under experiment could not be induced, on the fairly wide selection of foods offered, to consume the quantities of dry matter indicated for their respective live-weights by Wood's table. The highest values, obtained with a wide selection of dry foods, were of the order of 85-90 per cent of the expectations based on Wood's standards. With the standard American type of ration (maize and clover hay) and a common type of British ration (roots plus hay *ad lib.* with limited concentrates), the consumption fell to 72.5 per cent of the expectation based on Wood's data. Among the causes of depression of appetite the authors called special attention to the following: (1) protein-deficient rations; (2) rations containing an excessive proportion of roots.

It may be stated at once that the results of the feeding trials to be described in this paper afford a substantial confirmation of the Oxford findings. It should be emphasized, however, that these attempts to give numerical expression to the so-called "normal" appetites of sheep at different live-weights are essentially arbitrary. At best the values so obtained can merely serve for guidance when rationing sheep on the same type of ration as was employed in the determination of the standards. They have no absolute significance. It will be shown, for example, that the appetite of any given individual on a *constant* diet may vary very widely from day to day. Changes in the nature of the diet (e.g. the "balance" of the ration, its palatability and digestibility, the proportion of coarse fodder or succulent foods in the ration) may also be responsible for abrupt changes of the appetite of an individual animal. Unfortunately the criterion of lb. dry matter is not a really satisfactory expression of the true bulk of a ration, since it makes no distinction, from the standpoint of bulkiness, between a pound of dry matter in highly digestible concentrates and in such genuinely bulky foods as coarse fodders and roots.

Not only may the appetite of an individual be affected by dietary factors, but such considerations as the prevailing temperature and the amount of freedom for exercise may also come into play. If then it is difficult to give a satisfactory measure of the appetite of any *one* sheep at any given live-weight, the difficulty is greatly magnified when the problem becomes one of securing a measure of appetite that can be applied to sheep in general. Here the questions of breed and idiosyncrasy introduce further complications. It is essential to remember, therefore, that such figures as are obtained can have no absolute significance, but are of value in so far as they furnish useful guidance when computing rations.

#### *The present trials*

A row of out-door pens was constructed for the purpose of the feeding trials on a light, well-drained soil on the University Farm at Cambridge. Each pen measured 20 by 5 ft. and was designed to house one animal, adequate room for walking up and down being thus provided. The dividing partitions between contiguous pens consisted of hurdles, and by this means the animals under experiment were able to retain the desired sense of companionship. The feeding end of every pen was securely boarded and roofed for a distance of about 4 ft. in such a way as to protect the food in the troughs from rain and also to afford shelter to the sheep during periods of inclement weather.

In order that the feet of the sheep should remain clean and dry, and also to facilitate the daily cleaning out of the pens, the floors of the latter were covered by well-fitting creosoted boards. A plentiful supply of drinking water was kept in each pen. The general arrangements therefore permitted of the carrying out of the feeding trials under out-door conditions of temperature comparable with those under which sheep are folded during winter.

The trials were conducted during the winters of 1933-4, 1934-5 and 1935-6. The experimental animals during the first winter consisted of nine pure-bred Suffolk wethers. Five of these were about 7 months old at the beginning of the experiment, whereas the remaining four were mature sheep of  $2\frac{1}{2}$  years. Ten periods of feeding were carried out, during which the dry matter in the daily ration and in the food residues was determined for each animal. In this way determinations of daily dry-matter consumption were made on the following diets: lucerne hay of medium quality fed both in the chaffed and in the long condition; unchaffed lucerne hay of very good quality; lucerne hay and swedes; lucerne hay, swedes and balanced concentrates; rye grass-sainfoin hay, swedes and concentrates; hay, marrow-stem kale and concentrates; hay, raw potatoes and concentrates. Both swedes and potatoes were fed in the sliced condition. Hay was always given *ad lib*. Where kale, swedes or potatoes were included in the ration, the amounts of these were also adjusted to supply rather more than the sheep could eat. The concentrate part of the ration, however, was restricted to such amounts as would be fed in farming practice.

Records were kept of the live-weights of the sheep throughout the trial, every endeavour being made to ensure that the fleeces were reasonably dry at the times of weighing. At certain stages the weighings were made on three successive mornings in order to secure a more reliable check on the progress of the animals.

Five wethers (by Suffolk ram out of Cheviot  $\times$  Border Leicester ewes) were used in the second investigation during the winter of 1934-5. They had been weaned on 27 June and were almost exactly 3 months old at the beginning of the feeding trials in early July. The diet in the first feeding period was composed of meadow hay, green lucerne and balanced concentrates, but later in the year, when kale was available, the lucerne was replaced by either marrow-stem or thousand-head kale.

In the final trials, during the winter of 1935-6, seven sheep (four wethers and three females) of the same breeding as the foregoing were employed. They had been weaned on 5 August and were about 5 months

old when the experiment was begun in early September. The rations were made up from hay, marrow-stem kale and balanced concentrates, but in the final feeding period the kale was replaced by sliced mangolds.

### *Results of appetite tests*

A specimen summary of the results for one sheep during a single feeding period is given in Table II.

Table II. *Record of food consumption by sheep 18 (live weight = 91.3 lb.) on a diet of chaffed meadow hay, marrow-stem kale and concentrates\* (October 1935)*

Day	gm. dry matter in ration				gm. dry matter in residues				gm. dry matter consumed			
	Hay	Conc.	Kale	Total	Hay	Conc.	Kale	Total	Hay	Conc.	Kale	Total
1	549	325	900	1774	357	—	138	495	192	325	762	1279
2	549	325	890	1764	365	—	244	609	184	325	646	1155
3	549	325	1152	2026	193	—	198	391	356	325	954	1635
4	549	325	1020	1894	130	—	290	420	419	325	730	1474
5	549	325	1128	2002	323	—	249	572	226	325	879	1430
6	549	325	1032	1906	78	—	238	316	471	325	794	1590
7	549	325	1092	1966	263	—	227	490	286	325	865	1476
8	549	325	1020	1894	263	—	242	505	286	325	778	1389
9	549	325	1080	1954	296	—	235	531	253	325	845	1423
10	549	325	1032	1906	—	—	252	252	549	325	780	1654
11	549	325	1032	1906	210	—	317	527	339	325	715	1379
12	549	325	996	1870	287	—	220	507	262	325	776	1363
13	549	325	876	1750	—	—	190	190	549	325	686	1560
14	549	325	960	1834	59	—	273	332	490	325	687	1502

\* Consisting of 2 parts by weight of crushed oats and 1 part of decorticated ground nut cake.

It will naturally not be feasible to include in this paper the day-by-day records of consumption for all the sheep during the whole course of the investigation. In the Appendix are given, however, the average amounts of food, in terms of dry matter, consumed per day by each sheep during the different feeding periods, these figures being essential to the inquiry relating to the maintenance requirements of sheep (see Section II).

The specimen data in Table II serve to emphasize the variability from day to day of the appetite of an individual sheep even when the diet remains unaltered. They also call attention to the large amount of routine work and analysis involved in this type of experiment, a circumstance that explains why only small numbers of animals could be employed at one time.

An inspection of the tables in the Appendix reveals a number of interesting features connected with the influence of the nature of the diet on the appetite of sheep. The wide range of variation of appetite among sheep subsisting on a common diet will be noted. In period 7 (1933-4),

for example, the results ranged from a dry-matter consumption by sheep 6 amounting to about 103 per cent of the value predicted from Wood's standards down to about 78 per cent in the case of sheep 3. The effect of idiosyncrasy is thus very evident.

The results of the first feeding trial show that changing from a diet of chaffed lucerne hay to one of the same hay fed in the long condition occasioned a depression of appetite in the case of every animal. The average depression was 0.42 lb. of dry matter per day, amounting to 11.4 per cent of the mean daily dry-matter consumption in the first of the periods under consideration. An improvement in the quality of the lucerne hay (fed unchaffed) led in all cases to an increase in the amount consumed (period 3).

The introduction of swedes into the ration consisting of the poorer quality of lucerne hay led to a serious depression of appetite in one case only, namely, sheep 7. With sheep 1 an actual improvement was noted, but the general tendency was in the direction of depression (compare figures in final columns for periods 1, 4 and 5). The further addition of the concentrate allowance to the ration was followed by a stimulation of the appetite of every sheep under experiment (compare periods 5 and 6). Analysis of the results for periods 6, 7, 8 and 9 leads to the belief that the substitution of swedes by kale did not materially affect the appetites of the sheep. The replacement of swedes by raw potatoes, however, led to a very distinct fall in the amount of dry matter consumed per day. The period of measurement, however, was only 9 days, and too much significance should therefore not be attached to this finding.

The case of sheep 14 in the 1934-5 trials is of interest. This lamb was the lightest of the group at the beginning of the experiment in July. At the end of the trial in the following March, however, it had become the heaviest animal, a change to be attributed to its liking for meadow hay, which during this year was of very good quality.

The results for dry-matter consumption per day are expressed in every case as percentages of the amounts that would have been predicted, on the basis of live-weight, from Wood's standards. They are shown in the final column of the tables in the Appendix. In Table III they are arranged in relation to live-weight and without reference to the type of feeding. The results for the period in which potatoes were included in the diet, however, are omitted from the groupings, as well as those for the final period in 1934-5, when the sudden omission of kale from the ration of hay, concentrates and kale led to a pronounced drop in dry-matter consumption.

Table III. *Summarizing the results for mean daily dry-matter consumption, expressed as percentages of the predicted consumption and grouped according to live-weight of sheep*

Live-weight lb.	Appetite as percentage of amount predicted from Wood's standards	Mean %
60-65	82.4	82.4
65-75	{ 70.3, 74.0, 77.0, 77.1, 78.8 80.4, 80.8, 81.1, 81.5, 84.0, 84.9, 86.4	{ 79.7
75-85	{ 72.9, 76.1 81.5, 82.4, 84.2, 85.0, 85.8, 88.0, 88.2 92.1, 93.1, 93.5 102.3, 103.5	{ 87.8
85-95	{ 75.6, 76.5, 77.9, 78.1, 78.3 86.2, 86.6, 87.4, 89.3, 89.6 97.0, 98.8 101.2, 103.7, 105.5	{ 88.8
95-105	{ 74.2, 78.6 80.0, 80.0, 83.1, 83.4, 83.5, 83.8, 84.3, 85.5, 86.9, 88.2, 88.9 90.2, 90.5, 91.0, 91.3, 92.6, 93.4, 94.7, 96.5, 97.4 102.9	{ 87.9
105-115	{ 69.1 72.1, 74.7, 75.3, 77.4, 77.8, 78.3 80.3, 80.6, 81.7, 82.5, 83.1, 83.7, 83.9, 84.7, 84.8, 85.0, 85.1, 85.6, 86.6, 87.1, 87.6, 87.7, 87.9, 88.0, 88.3, 88.8 90.0, 90.0, 96.5 100.5, 101.9	{ 84.6
115-125	{ 71.8, 77.7, 78.4, 79.3, 79.5 80.0, 80.3, 81.0, 81.7, 81.8, 83.1, 83.2, 83.3, 83.9, 84.9, 86.1, 86.8, 87.3, 87.6 91.5, 91.8, 92.3, 93.0, 93.4, 94.6, 96.9	{ 85.0
125-135	{ 70.4, 73.4, 73.4, 74.6, 75.2, 79.3 81.0, 81.2, 82.3, 86.2, 88.4 94.8, 95.2	{ 81.2
135-145	{ 77.0 82.2 99.8, 90.5	{ 87.4
145-155	No results	—
155-165	79.6	79.6
165-175	{ 82.0, 83.0, 85.1 90.0, 90.5, 91.6, 92.4	{ 87.8
175-185	{ 84.8, 87.4 90.9, 96.4 103.8	{ 92.7
185-195	{ 84.1 93.4 102.0, 102.9	{ 95.6
195-205	{ 87.6 108.0	{ 97.8

The appetite data in Table III are notable for the wide range of the values, which vary from 69.1 to 108 per cent of the predicted appetites.



The results are entirely consistent with the observations of Prof. Watson *et al.* (4). In the vast majority of the cases, the dry-matter consumption fell below the standards formulated by Prof. Wood. In eighty-six out of 159 cases the consumption of dry matter lay between 80 and 90 per cent of the predicted value, in thirty-four cases between 69 and 80 per cent and in twenty-seven cases between 90 and 100 per cent. In the twelve cases when appetite actually exceeded the standards, two good feeders (sheep 4 and 6) were responsible for two and four cases respectively, while four of the remaining six cases were grouped together in a single feeding period, namely, when sheep 10, 11, 12 and 14 were brought on to kale in September 1934.

There is some evidence that the heavier sheep displayed, on an average, the better appetites relative to the standards, the mean of twenty-three results corresponding with live-weights from 135 to 205 lb. being 90.6 per cent (general average of all results = 86.0 per cent). Since sheep are not usually fed to such live-weights, it may be desirable to exclude the results for the heavy sheep from the final average. If this course is followed, the average of the 136 results for sheep varying from 60 to 135 lb. live-weight becomes 85.2 per cent.

The appetite of the 100 lb. sheep is, according to Wood's standards, 3.4 lb. of dry matter per day. If this value be corrected on the basis of the conclusions from the present investigation, the standard appetite becomes  $3.4 \times 85 \div 100 = 2.9$  lb. of dry matter, a value in reasonably good agreement with Watson's figure 2.6–2.7 lb. and with that of Henry and Morrison, namely 2.7–3.1 lb. Bearing in mind that such appetite standards are merely put forward for furnishing guidance when supervising the rationing of sheep, the writers consider it sufficient, in revising Wood's obviously too high standards, to multiply them throughout by the factor 0.85. This accordingly has been done in Table VIII.

## SECTION II. MAINTENANCE AND PRODUCTION REQUIREMENTS OF SHEEP AT DIFFERENT LIVE-WEIGHTS

A disquieting lack of agreement exists among the values that have been given at various times by different authorities for the maintenance starch-equivalent requirement of sheep. The older work has been summarized by Armsby<sup>(5)</sup> in the manner shown in Table IV.

It may be concluded from Table IV that the maintenance requirement of the standard 100 lb. sheep, according to the earliest work on the subject, is 791 Cal. of net energy, or 0.74 lb. of starch equivalent, per day.

Kellner (6), at a somewhat later date, gave the starch-equivalent requirement for maintenance (including wool growth) as 0.83 lb. per day per 100 lb. live-weight for sheep of the big breeds and 0.90 lb. for sheep of the smaller breeds.

Table IV. *Summary of older work on maintenance requirements of sheep*

	Net energy per 100 lb. live-weight therms
Respiration experiments:	
Henneberg and Stohmann (1867-8)	0.779
Henneberg, Fleischer and Müller (1872)	0.781
Kellner	0.611
Hagemann (1899)	0.705
Average	0.719
Live-weight experiments:	
Wolff (1871)	0.863
Wolff (1893)	0.863
Carlyle and Klienheinz	0.832
Average	0.853
Average of all values	0.791

On the basis of the figures in Table IV, and on the assumption that the maintenance starch-equivalent requirements are proportional to the two-thirds power of the live-weight, Armsby (5) suggests that the values in Table V represent the requirements for maintenance of sheep at different live-weights.

Table V. *Maintenance starch-equivalent requirements of sheep according to Armsby*

Live-weight of sheep (lb.)	60	80	100	120	140	160	180	200
Maintenance starch equivalent (lb.)	0.52	0.63	0.74	0.83	0.93	1.02	1.09	1.17

The results of more recent work, however, have thrown doubt on the conclusions drawn from these earlier investigations and have led to the belief that the starch equivalent for maintenance in sheep is considerably higher than has been assumed hitherto. Woodman *et al.* (7) utilized the results from digestion and balance trials on young, leafy grass to calculate the maintenance requirements of adult sheep and obtained the value 1.29 lb. of starch equivalent per day for the 100 lb. sheep. In subsequent work on similar lines (8) the value 1.18 lb. was arrived at, the mean value for the two investigations being 1.24 lb. The possibility was kept in mind, however, that this high result might have been connected with a stimu-

lation of metabolism associated with the protein-rich character of the grass diet on which the sheep subsisted during the trials.

Wood & Capstick<sup>(9)</sup>, by the statistical treatment of a large number of data from digestion trials on sheep subsisting on a wide range of diets, arrived at a figure in close agreement with the foregoing, namely, 1.26 lb. of starch equivalent for the standard 100 lb. sheep. It is on the basis of this figure that the starch-equivalent values for maintenance in Prof. Wood's standards (see Table I) have been calculated, the proportionality between maintenance requirements and the two-thirds power of the live-weight having been used for this purpose. These values, however, are not accepted by Prof. Watson *et al.*<sup>(3)</sup>, who, following an analysis of the results of past sheep-feeding trials, maintained that the use of Kellner's figure for maintenance (0.83 lb. of starch equivalent per 100 lb. live-weight) enabled the live-weight increase to be predicted with greater accuracy than was the case when the higher figure for maintenance (1.26 lb. per 100 lb. live-weight) was used.

The recent work of Ritzman & Benedict<sup>(10)</sup> on the energy metabolism of sheep has supplied evidence strongly supporting the higher figure. In six group experiments with adult males (fed on hay and roots) the range of standard metabolism, while standing, and when on maintenance diets and in moderate nutritive condition, was 29.2–35.7 Cal. per kg. live-weight, with an average value of 31.9 Cal. per kg. In adult ewes on maintenance diets, the range was 33–35.3 Cal. per kg. live-weight, with an average value of 33.6 Cal. The results are stated on the basis of 24 hours. The figures given for adult sheep apply closely also to sheep varying from 70 to 140 lb. live-weight. The authors state that their results point to a much higher metabolism in sheep than would be predicted on the basis of the old investigations of Henneberg, Kellner, Hagemann, Armsby, etc.

The standard metabolism is the heat evolution of the animal, quietly standing, 24 hours after food ingestion. This is 17 per cent higher than the true basal metabolism, two corrections being necessary, namely, one of 2 per cent for the stimulus due to food which still prevails after 24 hours, and another of 15 per cent for the effort of standing. The average basal metabolism of the sheep is therefore about 28 Cal. per 24 hours per kg. of body weight, or 1.19 lb. of starch equivalent per day per 100 lb. live-weight. This represents, therefore, the amount of starch equivalent that should be provided in the ration of a 100 lb. sheep for the mere maintenance of life. Since it makes no provision for the energy expended on voluntary muscular activity during the day, it must be regarded as a minimum figure to which a further small amount of starch equivalent

should be added to give the true maintenance requirement. In the light of this inquiry, therefore, the value for maintenance based on the old investigations (0.74 lb. of starch equivalent per 100 lb. live-weight) seems impossibly low, while the higher figure of 1.26 lb. takes on an aspect of probability.

Further confirmation of the higher value is forthcoming from the results of Tomme's<sup>(11)</sup> recent respiration studies on sheep. This investigator concludes that, under the conditions of his laboratory experiments, the maintenance requirement of the 100 lb. sheep is 1.15 lb. of starch equivalent per day, and that this figure should be increased by at least 10 per cent for sheep when fed in pens on the farm. The requisite 10 per cent increase brings Tomme's standard to 1.26 lb. of starch equivalent for the 100 lb. sheep, a result in striking agreement with the value suggested by Wood & Woodman.

It may be concluded, therefore, that recent scientific work affords strong support to the new figure for the maintenance starch equivalent of the 100 lb. sheep, namely, 1.26 lb. per day. It will be of interest to ascertain whether this value is consistent with the results of the present feeding trials. It is desirable to emphasize, however, that feeding trials having as their object the determination of live-weight increase cannot legitimately be employed to *establish* the magnitude of maintenance requirements. This objective should be reached by way of feeding trials conducted in respiration chambers. When, however, the feeding trials are carefully controlled, as in the present case, the results may usefully be employed to *check* the reliability of maintenance data already established on the basis of respiratory studies, particularly when a decision has to be made between two such widely differing standards as have been put forward for maintenance in sheep.

The results in Table VI of the feeding trial with sheep 15, 17, 18, 19, 20 and 21 over a 14 weeks' period, from 11 September to 17 December 1935, enable the actual live-weight increases to be compared with predictions based both on the old Armsby standards for maintenance (Table V) and the new standards (Table I). The results for each animal have been obtained *by summing the separate computations for each short period within the whole period of the trial*, a procedure leading to a more accurate estimate of maintenance and production requirements than is possible by making a single calculation on the basis of the mean live-weight over the entire period of the trial.

In commenting on the results in Table VI it should be kept in mind that exact agreement between actual and predicted live-weight increase

cannot be expected in the case of individual sheep. The standard values for maintenance, like those for appetite, have no absolute significance. They are average values derived from experiments on a limited number of animals. It may not be assumed, therefore, that these standard mean values are applicable with accuracy to every individual in a group of sheep, where, because of the factor of idiosyncrasy, the actual individual maintenance requirements may vary over a fairly wide range. Only when a large number of animals are employed in a feeding trial may it sometimes happen that the standard mean value coincides with the mean of the actual maintenance requirements of the individual sheep under experiment.

Table VI. *Summary of results for sheep 15, 17, 18, 19, 20 and 21 during 14 weeks' feeding period, 11 September to 17 December 1935*

Sheep no.	Range of live-weight lb.	Maintenance requirements in lb. starch equivalent		lb. starch equivalent consumed				lb. starch equivalent available for live-weight increase		Predicted (6) lb. live-weight increase		Actual lb. live-weight increase
		New stan- dards (1)	Old stan- dards (2)	lb. starch equivalent consumed				New stan- dards	Old stan- dards	New stan- dards	Old stan- dards	
				Hay (3)	Conc. (4)	Kale (5)	Total					
15	64-92	106.4	61.6	18.4	51.4	83.7	153.5	47.1	91.9	29.8	58.1	28.0
17	68-105	112.7	65.1	22.8	51.4	101.5	175.7	63.0	110.6	37.7	66.5	37.0
18	75-109	120.5	69.2	33.3	51.4	101.0	185.7	65.2	116.5	35.4	63.6	34.0
19	62-93	106.4	61.5	18.0	51.4	92.8	162.2	55.8	100.7	35.3	63.6	31.0
20	63-102	111.2	64.3	13.5	51.4	102.3	167.2	56.0	102.9	34.7	63.5	39.0
21	65-94	107.4	61.7	15.2	51.4	90.3	156.9	49.5	95.2	31.0	59.7	29.0
Totals for six sheep										203.9	375.0	198.0

(1) See Table I; (2) see Table V; (3) starch equivalent of chaffed meadow hay and rye grass-sainfoin hay = 46.7 and 46.0 on basis of dry matter (calculated from results of digestion trials); (4) starch equivalent, on basis of dry matter, of concentrate mixture = 73; (5) starch equivalent of kale as determined by digestion trials = 65.6 on basis of dry matter; value increased to 70 to allow for fact that kale residues left by sheep consisted of coarse part of stems; (6) production requirement per lb. live-weight increase taken as given in Table I.

In view of the foregoing considerations, therefore, it will be desirable to base conclusions on the results for all the sheep taken together rather than on the results for the individual animals. The total live-weight increase of the six sheep was 198 lb. The live-weight increase predicted on the basis of the new maintenance standards was 203.9 lb., a figure displaying remarkably close agreement with the experimental result. The use of the old Armsby standards, however, led to an impossibly high prediction, namely, 375 lb. Over a range of live-weight from 66.2 to 99.2 lb. (averages of the initial and final live-weights of the sheep under experiment) it may be claimed, therefore, that the results of this feeding trial furnish good support to the new standard for maintenance (1.26 lb. of starch equivalent per day for the 100 lb. sheep).

The results for the two periods of feeding with sheep 10, 11, 12, 13 and

14 during the winter of 1934-5, together with the results for sheep 1, 2, 3, 4 and 7 during the winter of 1933-4, are summarized in Table VII.

The results in Table VII for the first feeding period in 1934-5 (range of mean live-weight of sheep from 86.9 to 112.8 lb.) give further support to the revised standards for maintenance. Although the agreement between the predicted and observed total live-weight increase is not so close as in the case already considered, it is obvious that the new standards enable a much closer prediction to be made than the old. It should be noted that the result for sheep 11 is excluded from consideration, since this animal made unaccountably poor progress during this part of the experiment. The superiority of the new standards over the old as a means of enabling live-weight increase to be predicted is again evident from the results of the second period of feeding at the heavier levels of live-weight, and from the combined results of the two periods of feeding. The results of the 1933-4 trial also point distinctly to a decision in favour of the new standards.

It has already been pointed out that standard maintenance values are merely average data, from which the maintenance requirement of an individual animal may vary very considerably. They are by no means physiological constants that can be applied indiscriminately to all animals, or used as a basis for exact computations of live-weight increase in individual cases. Reliability of prediction can only be expected when forecasting the total live-weight increase of large groups of animals subsisting on rations of known starch equivalent. Bearing this in mind, and also the difficulty of ascertaining the true live-weight of sheep under out-door conditions in winter, it may be concluded that the agreement between the observed total live-weight gain of the relatively small number of sheep in the present trials and that predicted on the basis of the new maintenance standards is quite satisfactory. It is recognized, however, that a decision on this question by this type of experimentation would have been difficult but for the fact that the old and new standards for maintenance are so widely different (0.74 and 1.26 lb. of starch equivalent per day per 100 lb. live-weight).

The proposed revision of the feeding standards is shown in Table VIII. The standard maintenance value of 1.26 lb. of starch equivalent per day (rounded off to 9 lb. per week) is retained for the 100 lb. sheep, the values at other live-weights being computed on the basis of the proportionality between maintenance requirements and the two-thirds power of the live-weight. The figures for appetite have been reduced by multiplying Wood's values throughout by the factor 0.85 (see Section I).

Table VII. *Summary of results for sheep 10, 11, 12, 13 and 14 (1934-5) and for sheep 1, 2, 3, 4 and 7 (1933-4)*

Sheep no.	Range of live-weight lb.	Maintenance requirements in lb. starch equivalent		lb. starch equivalent consumed			lb. starch equivalent available for live-weight increase		Predicted lb. live-weight increase		Actual lb. live-weight increase	
		New standards	Old standards	Hay(1)	Conc.(2)	Kale(3)	Total	New standards	Old standards	New standards		Old standards
Feeding period, 24 Sept. to 3 Dec. 1934:												
10	88.5-118.5	91.0	52.7	35.1	39.9	72.6	147.6	56.6	94.9	28.7	48.0	30.0
11	87-106	87.7	50.7	34.4	39.9	73.5	147.8	60.1	97.1	30.9	50.1	19.0
12	90-115.5	90.0	52.0	37.7	39.9	72.8	150.4	60.4	98.4	30.5	49.6	25.5
13	91.5-115	90.3	51.9	38.5	39.9	59.9	138.3	48.0	86.4	24.4	43.8	23.5
14	77.5-102	82.1	47.3	40.8	39.9	55.1	135.8	53.7	88.5	31.0	51.1	24.5
							Totals for four sheep (omitting sheep 11)					
Feeding period, 7 Jan. to 25 Mar. 1935:												
10	117.5-144	115.8	68.1	30.0	66.6	70.6	167.2	51.4	99.1	19.0	36.4	26.5
11	110.5-134	112.3	65.7	28.0	66.6	71.3	165.9	53.6	100.2	21.4	39.7	23.5
12	117.5-136	114.3	67.0	27.6	66.6	70.3	164.5	50.2	97.5	19.2	37.2	18.5
14	110.5-146	114.6	67.4	48.5	66.6	69.2	184.3	69.7	116.9	26.5	44.0	35.5
							Totals for four sheep					
							Combined totals					
							200.7					
							157.3					
							349.8					
							207.5					
Feeding period, 2 Jan. to 21 Feb. 1934:												
1	107-127	69.9	40.6	29.8	19.3	50.0	99.1	29.2	58.5	12.1	24.4	20.0
2	106-121	69.0	39.9	26.2	19.3	54.8	100.3	31.3	60.4	13.8	26.5	15.0
3	105-114	66.8	38.6	19.6	19.3	52.8	91.7	24.9	53.1	11.6	24.7	9.0
4	115-131	72.1	42.1	30.8	19.3	59.9	110.0	37.9	67.9	14.9	26.8	16.0
7	113-131	70.4	40.9	25.6	19.3	54.4	99.3	28.9	58.4	11.3	23.3	18.0
							Totals for five sheep					
							63.7					
							125.7					
							78.0					

(1) Starch equivalent of dry matter of chaffed meadow hay (early-cut sample of excellent quality) used in 1934-5 trials = 50 (actual determination); chaffed lucerne hay and chaffed ryegrass-sainfoin hay in 1933-4 trials had starch equivalents of 45.6 and 46 respectively (actual determinations), both values being on dry-matter basis; (2) starch equivalent of dry matter of concentrate mixture = 90 in 1934-5 and 72 in 1933-4; (3) succulent food in 1933-4 trials was either kale or swedes (see Appendix); starch equivalent of kale = 70, of swedes = 63.5, both values being on basis of dry matter.

It is proposed in the new table to fix the maximum production requirement per lb. live-weight increase in the fat animal at 4 instead of  $4\frac{1}{4}$  lb. of starch equivalent. It is recognized that 1 lb. of live-weight increase in the form of pure, dry body fat would require a production allowance of 4 lb. of starch equivalent; and although on theoretical grounds the possibility is admitted of more than this allowance being required in certain circumstances for 1 lb. of live-weight increase in very fat animals, the scanty evidence available does not warrant the allowance being increased beyond 4 lb. for sheep at 170–200 lb. live-weight. The matter in any case is of academic significance, since in practice sheep are not pushed to such an extreme and uneconomic state of fatness.

Table VIII. *Revised table of sheep-feeding standards*

Live-weight lb.	Appetite, dry matter per week lb.	Maintenance requirement (per week)		Total protein equivalent required per week lb.	Production requirement per lb. live-weight increase, lb. starch equivalent
		lb. starch equivalent	lb. digestible protein		
60	14.5	$6\frac{1}{4}$	0.24	$1\frac{1}{2}$	$1\frac{1}{2}$
70	16.2	7	0.28	$1\frac{1}{2}$	$1\frac{1}{2}$
80	17.9	$7\frac{3}{4}$	0.32	$1\frac{3}{4}$	$1\frac{1}{2}$
90	19.1	$8\frac{1}{4}$	0.35	$1\frac{3}{4}$	$1\frac{3}{4}$
100	20.4	9	0.38	$1\frac{3}{4}$	2
110	21.7	$9\frac{1}{2}$	0.42	$1\frac{3}{4}$	$2\frac{1}{4}$
120	22.9	10	0.46	$1\frac{3}{4}$	$2\frac{1}{2}$
130	24.2	$10\frac{1}{2}$	0.50	$1\frac{3}{4}$	$2\frac{3}{4}$
140	25.5	11	0.54	$1\frac{3}{4}$	3
150	26.8	$11\frac{1}{2}$	0.58	$1\frac{3}{4}$	$3\frac{1}{4}$
160	28.0	12	0.62	$1\frac{3}{4}$	$3\frac{1}{2}$
170	28.9	$12\frac{1}{2}$	0.66	$1\frac{3}{4}$	4
180	29.8	13	0.70	$1\frac{3}{4}$	4
190	30.6	$13\frac{1}{2}$	0.74	$1\frac{3}{4}$	4
200	31.5	14	0.78	$1\frac{3}{4}$	4

Attention should be directed to a further difference between the original and the revised standards, namely, that no attempt is made in the new table to state the requirements of sheep below 60 lb. live-weight. This is due to the uncertainty surrounding the figures for the maintenance requirements of lambs, which were computed from the standard value for the 100 lb. sheep on the assumption that the basal metabolism per unit surface area of sheep is constant throughout life (12). This assumption, admittedly tentative because of the scanty evidence available at the time, is not borne out by the results of the more recent work of Ritzman & Benedict (10) on the energy metabolism of sheep. Comparison of the respiratory metabolism of sheep at different stages of growth and maturity has shown that "the youthful organism in sheep, as in all other



animal life that has been studied, has a specifically high metabolism. The energy requirements per kg. of body weight are extraordinarily high at birth and during the early part of the suckling period, but drop rapidly thereafter, and by the date of weaning at the end of the fourth month of life reach a level below which there is only a relatively small further decrease, due purely to advance in age".

It would manifestly be unsafe, in the light of these findings, to compute the maintenance requirements of sheep below 60 lb. live-weight on the assumption that the surface law is applicable to lambs. The care of lambs during the period they are with the ewes must remain, for the time being at any rate, a matter rather of the feeder's art than of the strict application of scientific knowledge.

#### SUMMARY

Feeding trials with sheep, subsisting out-of-doors on typical winter rations of known dry-matter and starch-equivalent content, have been carried out during the winters of 1933-4, 1934-5 and 1935-6. Records have been kept over these periods of the live-weights of the animals and their daily consumption of dry matter and starch equivalent.

It has been found that the standards of appetite, in terms of lb. dry matter, proposed by Prof. T. B. Wood are uniformly too high, a result in harmony with the findings of Prof. J. A. S. Watson and co-workers at Oxford. It is suggested that Prof. Wood's values should be multiplied throughout by the factor 0.85 in order to obtain reasonable measures of the appetites of sheep at different live-weights.

The data from the feeding trials have been used in an attempt to decide between the old and the recently proposed standards for the maintenance starch-equivalent requirement of the 100 lb. sheep, namely, 0.74 and 1.26 lb. of starch equivalent per day. The results point to the reliability of the higher figure, and it is shown that the results of recent work on the energy metabolism of sheep are in harmony with this conclusion.

A table embodying a revision of Prof. Wood's feeding standards for sheep is included in the paper.

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## APPENDIX

APPENDIX						Range of daily consumption of dry matter		Actual consumption as % of predicted
Sheep no.	Mean live-weight of sheep lb.	Mean dry matter consumed per day				Min. lb.	Max. lb.	
		Hay lb.	Conc. lb.	Swedes lb.	Total lb.			
I. 1933-4 feeding trials								
Period 1 (12 Oct. to 1 Nov.). Chaffed lucerne hay of medium quality:								
1	106.0	2.87	—	—	2.87	2.59	3.16	80.6
2	102.5	3.25	—	—	3.25	2.98	3.53	93.4
3	106.0	2.91	—	—	2.91	2.73	3.12	81.7
4	110.5	3.72	—	—	3.72	3.56	3.83	101.9
5	166.0	4.30	—	—	4.30	4.01	4.79	90.0
6	180.0	5.19	—	—	5.19	5.13	5.25	103.8
7	115.5	3.45	—	—	3.45	3.22	3.60	91.8
Period 2 (12 Nov. to 2 Dec.). Long lucerne hay of medium quality:								
1	107.0	2.77	—	—	2.77	2.60	3.11	77.4
2	104.0	3.05	—	—	3.05	2.94	3.16	86.9
3	107.5	2.59	—	—	2.59	2.41	2.79	72.1
4	111.5	3.12	—	—	3.12	2.93	3.27	85.0
5	171.5	4.00	—	—	4.00	3.66	4.48	82.0
6	175.0	4.09	—	—	4.09	3.70	5.29	83.0
7	115.0	3.14	—	—	3.14	2.80	3.39	83.7
Period 3 (2-11 Dec.). Long lucerne hay of very good quality:								
1	104.0	3.42	—	—	3.42	3.27	3.53	97.4
2	101.0	3.55	—	—	3.55	3.22	3.88	102.9
3	104.5	3.13	—	—	3.13	2.61	3.57	88.9
4	112.0	3.71	—	—	3.71	3.50	3.84	100.5
5	170.5	4.45	—	—	4.45	4.00	4.74	91.6
6	169.5	4.48	—	—	4.48	3.95	5.06	92.4
7	115.0	3.30	—	—	3.30	3.11	3.42	88.0
Period 4 (11-20 Dec.). Chaffed medium lucerne hay plus swedes:								
1	106.0	2.37	—	0.75	3.12	3.03	3.24	87.6
2	102.0	2.34	—	1.01	3.35	3.09	3.56	96.5
3	104.0	1.86	—	0.95	2.81	2.73	2.89	80.0
4	116.5	2.46	—	1.03	3.49	3.32	3.58	92.3
5	172.5	3.14	—	1.03	4.17	3.97	4.34	85.1
6	175.0	3.43	—	1.03	4.46	4.15	4.65	90.5
7	117.5	1.93	—	0.80	2.73	2.61	2.79	71.8
Period 5 (2-12 Jan.). Chaffed medium lucerne hay plus swedes:								
1	109.5	1.97	—	1.11	3.08	2.92	3.24	84.8
2	106.0	1.74	—	1.42	3.16	3.01	3.24	88.8
3	103.5	1.45	—	1.30	2.75	2.61	3.00	78.6
4	115.0	1.87	—	1.42	3.29	3.10	3.53	87.7
5	179.5	2.54	—	1.69	4.23	4.12	4.28	84.8
6	181.5	3.02	—	1.82	4.84	4.43	5.12	96.4
7	112.0	1.65	—	0.90	2.55	2.30	2.77	69.1

## APPENDIX (continued)

APPENDIX (continued)						Range of daily consumption of dry matter		Actual consumption as % of predicted
Sheep no.	Mean live-weight of sheep lb.	Mean dry matter consumed per day				Min. lb.	Max. lb.	
		Hay lb.	Conc. lb.	Swedes lb.	Total lb.			
Period 6 (12-23 Jan.). Chaffed lucerne hay plus swedes plus concentrates*:								
1	112.5	1.61	0.67	1.29	3.57	3.41	3.68	96.5
2	108.5	1.10	0.67	1.47	3.24	2.91	3.41	90.0
3	104.0	0.84	0.67	1.42	2.93	2.91	2.96	83.5
4	118.0	1.55	0.67	1.47	3.69	3.58	3.81	96.9
5	184.0	2.06	0.67	1.87	4.60	4.47	4.75	90.9
6	186.5	2.63	0.67	1.89	5.19	5.05	5.37	102.0
7	112.0	1.32	0.67	1.15	3.14	3.06	3.30	85.1
Period 7 (23 Jan. to 7 Feb.). Chaffed rye grass-sainfoin hay plus marrow-stem kale plus concentrates*:								
				Kale				
1	116.5	0.98	0.67	1.49	3.14	2.97	3.36	83.1
2	115.0	0.86	0.67	1.68	3.21	3.03	3.32	85.6
3	108.0	0.66	0.67	1.49	2.82	2.76	2.93	78.3
4	124.0	1.15	0.67	1.86	3.68	3.52	3.85	93.4
5	188.0	1.39	0.62	2.29	4.30	4.00	4.63	84.1
6	192.5	2.04	0.67	2.62	5.33	5.00	5.55	102.9
7	117.5	0.87	0.67	1.73	3.27	3.16	3.39	86.1
Period 8 (7-21 Feb.). Chaffed rye grass-sainfoin hay plus marrow-stem kale plus concentrates*:								
1	123.5	0.91	0.67	1.85	3.43	2.70	4.06	87.3
2	120.0	1.05	0.67	1.81	3.53	2.91	3.95	91.5
3	112.0	0.66	0.67	1.93	3.26	2.83	3.64	88.3
4	129.0	1.02	0.67	2.15	3.84	3.34	4.26	94.8
5	192.5	1.50	0.48	2.86	4.84	4.00	5.50	93.4
6	199.0	1.90	0.67	3.12	5.69	5.00	6.58	108.0
7	126.5	0.85	0.67	2.29	3.81	3.17	4.26	95.2
Period 9 (1-20 Mar.). Chaffed rye grass-sainfoin hay plus swedes plus concentrates*:								
				Swedes				
1	125.0	1.42	0.68	1.26	3.36	3.17	3.61	84.9
2	119.5	1.32	0.68	1.23	3.23	2.98	3.36	83.9
3	116.5	1.28	0.68	1.32	3.28	3.02	3.56	86.8
4	134.0	1.30	0.68	1.32	3.30	3.05	3.45	79.3
6	202.5	2.21	0.68	1.77	4.66	4.12	5.04	87.6
7	133.0	1.33	0.68	1.35	3.36	3.21	3.45	81.2
8	157.0	1.28	0.68	1.74	3.70	3.41	3.87	79.6
9	181.5	1.98	0.68	1.73	4.39	3.70	4.94	87.4
Period 10 (20-29 Mar.). Chaffed rye grass-sainfoin hay plus potatoes plus concentrates*:								
				Potatoes				
1	125.5	0.99	0.69	0.95	2.63	2.32	2.84	66.2
2	122.5	0.98	0.69	0.97	2.64	2.34	2.80	67.5
3	120.0	0.88	0.69	0.97	2.54	2.06	2.84	65.8
4	134.0	0.91	0.69	0.97	2.57	2.00	3.07	61.8
5	182.0	1.78	0.70	1.02	3.50	3.25	3.75	69.6
6	201.5	1.82	0.69	1.02	3.53	3.18	3.93	66.5
7	135.0	1.28	0.69	0.97	2.94	2.76	3.11	70.3
8	160.0	1.64	0.69	0.94	3.27	2.93	3.60	69.4
9	184.5	2.47	0.69	0.97	4.13	3.86	4.38	81.6

2½ parts by weight of crushed oats to 1 part of decorticated ground nut cake.

APPENDIX (*continued*)

Sheep no.	Mean live-weight of sheep lb.	Mean dry matter consumed per day				Range of daily consumption of dry matter		Actual consump- tion as % of predicted
		Hay lb.	Conc. lb.	Lucerne lb.	Total lb.	Min. lb.	Max. lb.	
II. 1934-5 <i>feeding trials</i>								
Period 1 (9-26 July). Chaffed meadow hay plus green lucerne plus concentrates*:								
10	77.4	0.80	0.40	1.03	2.23	1.78	2.50	76.1
11	69.3	0.91	0.40	0.95	2.26	1.82	2.75	84.0
12	74.0	0.76	0.40	1.07	2.23	1.87	2.54	78.8
13	73.5	0.61	0.40	1.25	2.26	1.77	2.66	80.4
14	65.4	0.55	0.40	0.96	1.91	1.28	2.50	74.0
Period 2 (24 Sept. to 8 Oct.). Chaffed meadow hay plus marrow-stem kale plus concentrates								
Kale								
10	91.0	1.47	0.58	1.31	3.36	3.11	3.80	103.7
11	89.3	1.44	0.58	1.22	3.24	2.91	3.55	101.2
12	92.0	1.54	0.58	1.32	3.44	3.11	3.65	105.5
13	92.3	1.47	0.58	1.11	3.16	3.00	3.29	97.0
14	80.0	1.58	0.58	0.91	3.07	2.80	3.30	102.3
Period 3 (9 Oct. to 18 Nov.). Chaffed meadow hay plus thousand-head kale plus concentrates:								
10	102.8	0.93	0.63	1.52	3.08	2.53	3.60	88.2
11	97.3	0.95	0.63	1.54	3.12	2.81	3.38	92.6
12	100.3	1.01	0.63	1.48	3.12	2.87	3.44	91.0
13	101.0	1.06	0.63	1.26	2.95	2.57	3.34	85.5
14	88.0	1.10	0.63	1.11	2.84	2.50	3.11	89.6
Period 4 (19 Nov. to 3 Dec.). Chaffed meadow hay plus thousand-head kale plus concentrates:								
10	115.3	0.75	0.70	1.54	2.99	2.61	3.65	79.5
11	104.5	0.62	0.70	1.65	2.97	2.45	3.64	84.3
12	111.0	0.82	0.70	1.67	3.19	2.78	3.65	87.1
13	112.0	0.86	0.70	1.22	2.78	2.16	3.23	75.3
14	97.8	0.96	0.70	1.39	3.05	2.66	3.48	90.2
Period 5 (7-21 Jan.). Chaffed meadow hay plus marrow-stem kale plus concentrates:								
10	120.5	0.76	0.82	1.49	3.07	2.59	3.57	79.3
11	115.3	0.71	0.82	1.48	3.01	2.71	3.52	80.0
12	119.5	0.75	0.82	1.58	3.15	2.74	3.54	81.8
14	114.0	1.02	0.82	1.44	3.28	2.74	3.73	87.9
Period 6 (22 Jan. to 8 Feb.). Chaffed meadow hay plus marrow-stem kale plus concentrates								
10	125.8	0.66	0.86	1.45	2.97	2.86	3.02	74.6
11	121.0	0.75	0.86	1.56	3.17	3.00	3.36	81.7
12	125.0	0.84	0.86	1.60	3.30	3.24	3.44	83.3
14	121.8	1.33	0.86	1.50	3.69	3.56	3.71	94.6
Period 7 (9-23 Feb.). Chaffed meadow hay plus thousand-head kale plus concentrates:								
10	130.8	0.44	0.89	1.70	3.01	2.69	3.31	73.4
11	124.5	0.51	0.89	1.77	3.17	2.65	3.51	80.3
12	128.8	0.52	0.89	1.44	2.85	2.42	3.44	70.4
14	129.5	0.93	0.89	1.68	3.50	3.00	3.94	86.2
Period 8 (24 Feb. to 10 Mar.). Chaffed meadow hay plus thousand-head kale plus concentrates:								
10	138.8	0.56	0.93	1.79	3.28	3.16	3.39	77.0
11	130.5	0.47	0.93	1.67	3.07	2.90	3.22	75.2
12	132.5	0.37	0.93	1.73	3.03	2.87	3.18	73.4
14	139.5	0.93	0.93	1.66	3.52	3.40	3.87	82.2

\* Concentrates during 1934-5 consisted of 2 parts of flaked maize to 1 part of decorticated ground nut cake.

APPENDIX (*continued*)

APPENDIX ( <i>continued</i> )						Range of daily consumption of dry matter		Actual consumption as % of predicted
Sheep no.	Mean live-weight of sheep lb.	Mean dry matter consumed per day				Min. lb.	Max. lb.	
		Hay lb.	Conc. lb.	Kale lb.	Total lb.			
Period 9 (11-25 Mar.). Chaffed meadow hay plus concentrates:								
10	138.8	1.53	1.35	—	2.88	2.66	3.07	67.6
11	130.5	1.21	1.35	—	2.56	2.25	2.90	62.7
12	132.5	1.12	1.35	—	2.47	2.29	2.73	60.0
14	139.5	2.10	1.35	—	3.45	2.82	3.83	80.6

III. 1935-6 *feeding trials*

Period 1 (11-24 Sept.). Chaffed meadow hay plus marrow-stem kale plus concentrates\*:

15	66.0	0.57	0.60	0.95	2.12	1.68	2.60	81.5
17	70.3	0.85	0.60	0.90	2.35	1.93	2.90	86.4
18	79.8	1.16	0.60	0.79	2.55	1.97	2.86	85.0
19	64.3	0.68	0.60	0.82	2.10	1.82	2.47	82.4
20	65.8	0.48	0.60	0.74	1.82	1.50	2.14	70.3
21	66.5	0.56	0.60	0.95	2.11	1.79	2.38	80.8

Period 2 (25 Sept. to 15 Oct.). Chaffed meadow hay plus marrow-stem kale plus concentrates:

15	71.3	0.57	0.64	1.02	2.23	2.18	2.26	81.1
17	76.0	0.56	0.64	1.18	2.38	2.16	2.50	82.4
18	86.3	0.89	0.64	1.19	2.72	2.49	2.83	86.6
19	70.3	0.51	0.64	1.16	2.31	2.22	2.45	84.9
20	73.8	0.27	0.64	1.26	2.17	2.15	2.21	77.0
21	72.5	0.40	0.64	1.11	2.15	2.08	2.20	77.1

Period 3 (16-29 Oct.). Chaffed meadow hay plus marrow-stem kale plus concentrates:

15	76.9	0.31	0.72	1.42	2.45	1.88	3.09	84.2
17	82.0	0.36	0.72	1.75	2.83	2.58	3.21	93.1
18	91.3	0.76	0.72	1.72	3.20	2.54	3.64	98.8
19	77.0	0.29	0.72	1.71	2.72	2.56	2.95	93.5
20	82.3	0.22	0.72	1.87	2.81	2.43	3.11	92.1
21	78.5	0.20	0.72	1.62	2.54	2.20	3.02	85.8

Period 4 (30 Oct. to 12 Nov.). Chaffed rye grass-sainfoin hay plus marrow-stem kale plus concentrates:

15	81.6	0.47	0.72	1.28	2.47	1.55	2.87	81.5
17	88.0	0.49	0.72	1.56	2.77	2.16	3.30	87.4
18	97.8	0.94	0.72	1.54	3.20	2.57	3.88	94.7
19	83.0	0.46	0.72	1.52	2.70	1.93	3.09	88.2
20	88.7	0.44	0.72	1.69	2.85	2.30	3.30	89.3
21	81.5	0.31	0.72	1.18	2.21	1.48	2.90	72.9

Period 5 (13 Nov. to 3 Dec.). Chaffed rye grass-sainfoin hay plus marrow-stem kale plus concentrates:

15	87.0	0.27	0.80	1.31	2.38	2.29	2.47	75.6
16	79.5	0.49	0.83	1.31	2.63	2.50	2.76	88.0
17	96.0	0.40	0.80	1.60	2.80	2.74	2.84	83.8
18	103.0	0.41	0.80	1.69	2.90	2.72	3.05	83.1
19	88.5	0.24	0.80	1.45	2.49	2.40	2.60	78.3
20	95.5	0.24	0.80	1.60	2.64	2.60	2.66	80.0
21	87.0	0.31	0.80	1.35	2.46	2.36	2.63	78.1

\* Concentrates during 1935-6 trials consisted of 2 parts of crushed oats to 1 part of decorticated ground nut cake.

APPENDIX (*continued*)

Sheep no.	Mean live-weight of sheep lb.	Mean dry matter consumed per day				Range of daily consumption of dry matter		Actual consump- tion as % of predicted
		Hay lb.	Conc. lb.	Kale lb.	Total lb.	Min. lb.	Max. lb.	
Period 6 (4-17 Dec.). Chaffed rye grass-sainfoin hay plus marrow-stem kale plus concentrates:								
15	91.0	0.25	0.83	1.40	2.48	1.55	3.15	76.5
16	85.0	0.38	0.83	2.01	3.22	2.83	3.86	103.5
17	103.0	0.35	0.83	1.98	3.16	2.66	3.61	90.5
18	107.0	0.30	0.83	1.97	3.10	2.08	3.80	86.6
19	92.0	0.21	0.83	1.50	2.54	1.83	3.43	77.9
20	100.5	0.19	0.83	1.85	2.87	2.44	3.41	83.4
21	92.5	0.20	0.83	1.79	2.82	2.44	3.39	86.2
Period 7 (22 Jan. to 4 Feb.). Chaffed meadow hay plus marrow-stem kale plus concentrates:								
15	105.3	0.44	0.90	1.66	3.00	2.57	3.65	84.7
16	101.5	0.33	0.90	1.93	3.16	2.64	3.80	91.3
17	117.5	0.46	0.90	1.97	3.33	2.85	4.00	87.6
18	119.5	0.40	0.90	1.82	3.12	2.44	3.50	81.0
19	105.8	0.21	0.90	1.65	2.76	2.28	3.32	77.8
20	114.0	0.22	0.90	2.01	3.13	2.57	3.76	83.9
21	105.0	0.15	0.90	1.57	2.62	2.17	3.10	74.2
Period 8 (5-24 Feb.). Chaffed meadow hay plus marrow-stem kale plus concentrates:								
15	113.5	0.37	0.90	1.80	3.07	2.96	3.16	82.5
16	108.5	0.31	0.90	1.79	3.00	2.83	3.08	83.1
17	129.0	0.58	0.90	2.10	3.58	3.53	3.62	88.4
18	130.5	0.41	0.90	2.05	3.36	3.14	3.54	82.3
19	113.5	0.22	0.90	1.66	2.78	2.59	2.96	74.7
20	124.0	0.22	0.90	1.97	3.09	2.95	3.29	78.4
21	115.0	0.23	0.90	1.88	3.01	2.79	3.33	80.3
Period 9 (25 Feb. to 3 Mar.). Chaffed meadow hay plus mangolds plus concentrates:								
				Mangolds				
15	121.0	0.12	0.90	2.59	3.61	3.35	3.73	93.0
16	115.0	0.14	0.90	2.33	3.37	3.30	3.45	90.0
17	138.0	0.53	0.90	2.80	4.23	4.08	4.33	99.8
18	137.5	0.28	0.90	2.65	3.83	3.62	4.21	90.5
19	117.0	0.15	0.90	2.11	3.16	2.86	3.40	83.2
20	131.5	0.06	0.90	2.36	3.32	3.10	3.58	81.0
21	120.0	0.08	0.90	2.02	3.00	2.25	3.35	77.7

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## SHEEP NUTRITION

### II. DETERMINATIONS OF THE AMOUNTS OF GRASS CONSUMED BY SHEEP ON PASTURAGE OF VARYING QUALITY

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IN order that the nutrition of grazing animals may be studied from a scientific standpoint, it is necessary to have information on the following matters: (1) the requirements of the animals in terms of starch equivalent and digestible protein; (2) the capacity of pasture to supply these requirements; (3) the amount of grass consumed by the animals, so that the supplementary requirements (if any) may be ascertained; (4) the energy expended in grazing.

Information respecting the nutritive value of pasturage under different systems of grazing has already been obtained from the series of pasture investigations carried out in this Institute<sup>(1)</sup>. For a statement of the maintenance and production requirements, in terms of starch equivalent and digestible protein, of sheep at different live-weights, the reader is referred to the immediately preceding paper in this issue of the *Journal*<sup>(2)</sup>. In respect of the energy expended by animals in walking about on pasture and in biting off the herbage, no evidence from experimental inquiry is available. It has been assumed that such energy expenditure is conditioned by the density of the herbage, and estimates have been made, in terms of starch equivalent, of the energy used up by cattle when grazing pastures of differing quality<sup>(3)</sup>. Such estimates, however, are not based on actual measurement.

Neither is there any trustworthy information about the appetites of sheep when grazing freely on pasture. Owing to the difficulties involved in attempts to secure direct measurements of appetite (e.g. by tethering experiments), it has been the custom to assume that the amount of dry matter consumed per day in the form of herbage is the same as would be eaten by sheep of the same live-weight when subsisting on winter rations. It will be shown in this paper, however, that this assumption is entirely unjustified.

## PLAN OF PRESENT INQUIRY

In view of the obvious difficulties attendant on any attempt to measure by direct means the amount of grass eaten by sheep on pasture, it was decided to adopt an indirect method depending primarily on the determination of the weight of faeces voided per day by the sheep when grazing the experimental pasture plots. If the average daily output of faeces by each sheep over a given period be known, and if during this period the digestibility of the herbage be determined by independent digestion trials on other sheep, it is then a simple matter to calculate the mean daily grass consumption of each sheep over the period of measurement.

For such a method of experiment, however, it must be possible to collect quantitatively the dung from each sheep while it is grazing the pasture. This difficulty was overcome by the use of the type of digestion harness employed by Dr S. J. Watson in his digestibility experiments at the Jealott's Hill Agricultural Research Station,<sup>1</sup> the faeces being collected in a special waterproof bag attached to the hindquarters of the animal by a light webbing harness. This bag could be opened and closed by means of a lightning fastener passing down its full length, and by this means the faeces could be removed quite simply without unfastening the harness or detaching the bag.

With grazing sheep provided with such equipment it is of the greatest importance to keep the hindquarters scrupulously clean with a view to warding off the attacks of flies and other insects. The wool on the hindquarters was clipped as closely as possible, and this part was cleansed every day with soap and water containing a little lysol, being smeared afterwards with a preparation composed of 2 drachms of creosote and 8 oz. of terebene made up to 20 oz. with olive oil. This lotion proved a very effective safeguard against the fly danger.

Provision was made for shade on hot, sunny days and ample drinking-water was always available. The risk of "scouring", which would have rendered collection of the faeces impossible, was averted by giving each sheep an allowance of 100 g. undecorticated cotton cake daily, a similar allowance being given also to the sheep on which the digestibility of the herbage was being measured. With such precautions the trials were carried out with the greatest smoothness, and it is clear that this

<sup>1</sup> The writers take this opportunity of thanking Dr Watson for his help in this connexion. The suitability of this type of harness for the particular purposes of the present investigation contributed in no small measure to the success of the experiments.



technique offers great advantages in the study of the general principles underlying the nutrition of grassland sheep. It is intended to extend these preliminary investigations in this direction.

#### GRAZING TRIALS DURING THE 1934 SEASON

The trials were carried out on the light-land pasture on which the earlier Cambridge pasture investigations had been made<sup>(4)</sup>. The main plot was divided by means of hurdles into five subplots, each measuring 210 by 109 links. Nine pure-bred Suffolk wethers were used in the experiments, sheep 1, 2, 3, 4 and 7 being 13–14 months old, while sheep 5, 6, 8 and 9 were nearly 3 years of age.

Beginning on 17 April, sheep 1, 2, 8 and 9 were allowed to graze down the herbage on the first subplot over a period of 4 days. They were then turned on to the second subplot for a similar period and from thence, in succession, on to the three remaining subplots. At the end of this preliminary period, the four sheep were returned to the first subplot, which was now covered with an abundant leafy growth of mixed herbage,<sup>1</sup> in which perennial rye grass, rough-stalked meadow grass and cocksfoot were prominent. The experiment proper was begun at this stage (10 May). The sheep were passed from plot to plot in succession as in the preliminary period, the animals being moved on at a rate dictated by the necessity of always having an abundance of grass available for grazing. Each animal now received its daily allowance of 100 g. of undecorticated cotton cake. The faeces were collected in the manner already described, the bags being emptied twice daily. After weighing the faeces, representative aliquot portions were kept for determinations of dry matter and ash content.

The first grazing trial was of 15 days' duration, this period being divided into two subperiods of 8 and 7 days respectively. Contemporaneously with the grazing trials, determinations of the digestibility of the herbage were made on sheep 4 and 5. For this purpose, representative strips on the subplot being grazed at the time were cut by means of a motor lawn-mower, this procedure being followed day by day throughout the trial.

At the completion of the first grazing trial, the experiment was repeated, sheep 3, 4, 5, 6 and 7 being used this time for grazing and sheep 1 and 9 for the digestion trial. The results of the 1934 experiments are

<sup>1</sup> For details of the botanical character of the herbage on the experimental pasture, see reference (4).

summarized in Tables I and II. The Appendix to this paper should be consulted, however, for the detailed records of the investigation and the method of calculating the dry-matter consumption of the sheep.

Table I. *Composition and digestibility of pasture herbage in the different grazing periods (1934 experiment)*

	First grazing period		Second grazing period	
	Subperiod 1 (10-17 May)	Subperiod 2 (18-24 May)	Subperiod 3 (5-10 June)	Subperiod 4 (11-16 June)
On basis of dry matter:	%	%	%	%
Crude protein	19.14	16.73	14.10	12.97
Ether extract	7.95	6.98	6.14	5.38
N-free extractives	44.91	48.18	50.06	51.00
Crude fibre	18.99	19.37	21.18	21.56
Ash	9.01	8.74	8.52	9.09
Pepsin-HCl-soluble protein	15.46	13.50	11.09	10.00
Digestion coefficient of crude protein ( <i>in vitro</i> )	80.8	80.7	78.7	77.1
Digestion coefficient of organic matter (animal experiment)	81.7	80.4	73.7	71.8

Table II. *Mean daily appetites of sheep in lb. dry matter (1934 experiment)*

Sheep no. ... ..		Subperiod 1 (10-17 May)				Subperiod 2 (18-24 May)			
		1	2	8	9	1	2	8	9
Mean lb. dry matter consumed per day:									
Undecorticated cotton cake		0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Pasture herbage		4.47	4.47	4.80	6.04	4.87	4.98	4.73	5.94
Total		4.66	4.66	4.99	6.23	5.06	5.17	4.92	6.13
Mean live-weight of sheep in lb. over period 10-24 May		146	137	166	201				

Sheep no. ... ..		Subperiod 3 (5-10 June)					Subperiod 4 (11-16 June)				
		3	4	5	6	7	3	4	5	6	7
Mean lb. dry matter consumed per day:											
Undecorticated cotton cake		0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Pasture herbage		4.31	4.37	3.86	5.42	4.07	4.07	3.85	3.86	4.87	3.85
Total		4.50	4.56	4.05	5.61	4.26	4.26	4.04	4.05	5.06	4.04
Mean live-weight of sheep in lb. over period 5-16 June		138	151	184	215	152					

#### GRAZING TRIALS DURING THE 1935 SEASON

The four wethers used in the 1935 trials had been bred by crossing a Suffolk ram with Cheviot × Border Leicester ewes and were about 13 months old at the beginning of the experiment. The preliminary

procedure was the same as in 1934, but the actual measurements were begun about a fortnight later in the season, for the reason that the cold, dry spring had retarded growth on the pasture. An abundance of herbage was available, however, on 25 May, the first day of the appetite measurements. It was mainly leafy in character, but meadow foxtail was in flower and the flower-stems of perennial rye grass were emerging. The essential results of the 1935 grazing trial are shown in Tables III and IV. For a detailed summary of the observations, however, the reader is again referred to the Appendix.

Table III. *Composition and digestibility of pasture herbage*  
(1935 experiment)

	Subperiod 1 (25 May to 1 June)	Subperiod 2 (2-9 June)
On basis of dry matter:	%	%
Crude protein	18.98	19.52
Ether extract	3.92	4.61
N-free extractives	44.67	44.86
Crude fibre	20.32	21.15
Ash	12.11	9.86
Pepsin-HCl-soluble protein	15.67	15.68
Digestion coefficient of crude protein ( <i>in vitro</i> )	82.6	80.3
Digestion coefficient of organic matter (animal experiment)	79.4	78.5

Table IV. *Mean daily appetite of sheep in lb. dry matter*  
(1935 experiment)

Sheep no. ...	Subperiod 1 (25 May to 1 June)				Subperiod 2 (2-9 June)			
	10	11	12	14	10	11	12	14
Mean lb. dry matter consumed per day								
Uncorticated cotton cake	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Pasture herbage	3.21	4.39	4.30	4.66	3.69	4.50	4.09	4.24
Total	3.40	4.58	4.49	4.85	3.88	4.69	4.28	4.43
Mean live-weight of sheep in lb. over period 25 May to 9 June	150	140	150	155				

#### *Comments on Tables I, II, III and IV*

The computation of the dry-matter consumption of the grazing sheep is based on two assumptions that may be considered not entirely justifiable: (1) that the herbage cut by the lawn-mower is comparable, in respect of composition and digestibility, to the herbage eaten by the grazing sheep; (2) that the grazing sheep, on an unrestricted grass supply, digest the herbage to the same extent as the digestion sheep

receiving a restricted ration. It is not considered that the first objection is very serious. With pasturage 3-6 in. high, subjected to fairly intensive and therefore uniform grazing, it is a much simpler matter to cut a satisfactorily representative sample of herbage than would be the case with large and unevenly grazed plots. In the case of the second objection, it could be argued that the slight depression of digestibility that might possibly result from unrestricted feeding might in all probability be off-set by the stimulating influence on digestion of free exercise in the open air. It has been found, moreover, in earlier pasture trials that the size of the grass ration may be varied within wide limits without significantly affecting the extent to which it is digested by sheep. During the present trials, with the object of ensuring as far as possible the applicability of the digestion coefficients to the calculation of the grass consumption of the sheep on the plots, the digestion sheep were fed to the limit of their appetites as manifested in the confinement of the metabolism cages.

The digestion coefficient of the organic matter in the herbage was taken as constituting the best single index of digestibility. It was on the basis of this figure, therefore, that the food consumption of the grazing sheep was calculated (see Appendix). It was possible by this means to avoid any complications arising from accidental inclusion of soil in the samples of herbage cut for the digestion sheep, a circumstance that would have affected the validity of any calculations based on the digestion coefficient of the dry matter of the grass ration.

The results for the first grazing period of 1934 (see Table II) should reveal the maximum capacity of the sheep for consuming pasture grass, because of the excellent quality and high digestibility of the herbage at this early stage of the season (10-24 May). The results are compared in Table V with the values that would have been predicted on the basis of Wood's feeding standards for sheep<sup>(3)</sup>. Since continuous records, in terms of lb. dry matter, had been kept during the previous winter of the appetites of the sheep when subsisting on typical winter rations of hay, swedes and concentrates<sup>(2)</sup>, it is possible to include in Table V certain of the data so obtained for purposes of comparison.

The concentrate mixture fed in the winter trials consisted of 250 g. of crushed oats and 100 g. of decorticated ground nut cake. This was consumed completely on every day of the trial by all four sheep. They were permitted to eat in addition chaffed sainfoin-rye grass hay and sliced swedes up to the limit of appetite. It will be seen from Table V that the mean daily consumption of dry matter over this March period was definitely *smaller*, in the case of each sheep, than would have been

forecast on the basis of Wood's standards. Sheep 1 averaged approximately 85 per cent of the predicted dry-matter consumption, while sheep 2, 8 and 9 averaged 84, 80 and 88 per cent respectively.

Table V. *Appetites, in terms of lb. dry matter, of sheep on (a) pasture, and (b) a typical winter ration*

	Grazing trial (May 1934)				On typical winter ration (Mar. 1934)			
	Mean daily consumption on grass		Mean live-weight of sheep (10-24 May) lb.	Appetite as predicted from Wood's feeding standards lb. dry matter	Mean daily consumption (1-20 Mar.) lb. dry matter	Mean live-weight of sheep (1-20 Mar.) lb.	Appetite as predicted from Wood's feeding standards lb. dry matter	
	Subperiod 1 (10-17 May)	Subperiod 2 (18-24 May)						
	lb. dry matter	lb. dry matter						
Sheep 1	4.66	5.06	146	4.39	3.36	125	3.96	
Sheep 2	4.66	5.17	137	4.23	3.23	120	3.86	
Sheep 8	4.99	4.92	166	4.79	3.70	157	4.61	
Sheep 9	6.23	6.13	201	5.29	4.39	181	5.00	
Digestion coefficient of organic matter of grass	81.7 %	80.4 %						

This position, however, was reversed in the most striking manner when the appetites of the sheep were measured on the grazing plots during the May of the same year. In both subperiods of feeding all four sheep consumed a distinctly *higher* amount of dry matter per day than was predicted from Wood's standards. Averaging the results for the two subperiods, it is seen that sheep 1 ate approximately 111 per cent of the predicted amount, the corresponding figures for sheep 2, 8 and 9 being 116, 104 and 117 per cent respectively.

With the help of the results for the March trial it is possible to make an estimate of the amount of dry matter that the sheep would have consumed on the hay-swedes-concentrates ration when they had attained the live-weights at which the grazing trial in May was carried out. Such a calculation reveals that sheep 1, whilst subsisting on pasture, ate 1.13 lb. more dry matter per day than it would have eaten on the diet of hay, swedes and concentrates, the corresponding increases for sheep 2, 8 and 9 being 1.37, 1.13 and 1.53 lb. dry matter per day respectively. There is naturally an element of speculation in this last calculation, but the main conclusion is not in the least speculative, namely, that although the sheep, when tested on a typical winter ration, ate an amount of dry matter per day very definitely lower than that predicted from Wood's standards, yet when tested on good pasture, their appetites were distinctly higher than would have been expected from the standards.

The observed increase in appetite could not be attributed to the stimulating influence of being in the open air after a period of indoor confinement, since the sheep had been kept in pens on the same field throughout the entire duration of the winter-feeding trials. Neither could

it have been induced by the tonic effect of passing suddenly from winter-feeding to grazing, for the sheep had become well-accustomed to the grass, having been grazing the plots for more than 3 weeks before the actual measurements of appetite were begun. It must be ascribed, in the writers' opinion, to the high palatability of the young spring herbage.

The results support the contention that farm animals in general tend to over-eat when first put out to grass in spring, and that it is this excessive consumption of the succulent, protein-rich herbage that contributes largely to the "scouring" so frequently noted at this early stage of the grazing season. Advocates of restricting the hours of grazing at this period, particularly in the case of dairy cows after a winter of indoor-feeding, will find support for their arguments in the results of the foregoing experiment.

During the second grazing period, which began on 5 June, the quality of the herbage was not so good. There had been a marked increase in stemminess, and the pasture was obviously suffering from the effects of the prevailing hot, droughty weather. The extent of the deterioration may be gauged from the analytical data in Table I and from the finding that the digestion coefficient of the organic matter in the herbage had fallen from the initially high value of 81.7 to 73.7 per cent in subperiod 3 and 71.8 per cent in subperiod 4. That this decline in quality appears to have been accompanied by a corresponding deterioration in palatability is evident from the figures in Table VI, in which the mean daily consumption of dry matter by the grazing sheep (3, 4, 5, 6 and 7) is compared with the predictions from Wood's standards. Corresponding results for the same sheep when subsisting during the previous March on the diet of hay, swedes and concentrates are also included in the table.

Table VI. *Appetites, in terms of lb. dry matter, of sheep on (a) pasture, and (b) a typical winter ration*

	Grazing trial (June 1934)			On typical winter ration (Mar. 1934)		
	Mean daily consumption on grass		Mean live-weight of sheep (5-16 June)	Appetite as predicted from Wood's feeding standards lb. dry matter	Mean daily consumption (1-20 Mar.) lb. dry matter	Appetite as predicted from Wood's feeding standards lb. dry matter
	Subperiod 3 (5-10 June) lb. dry matter	Subperiod 4 (11-16 June) lb. dry matter				
Sheep 3	4.50	4.26	138	4.24	3.28	116
Sheep 4	4.56	4.04	151	4.51	3.30	134
Sheep 5*	4.06	4.05	184	5.06	—	—
Sheep 6	5.61	5.06	215	5.50	4.66	202
Sheep 7	4.26	4.04	152	4.53	3.36	133
Digestion coefficient of organic matter of grass	73.7	71.8				

\* No data available for this sheep in the case of the March experiment.

It is clear from the results in Table VI that the sheep, while subsisting during March on the winter diet of hay, swedes and concentrates, ate a distinctly smaller amount of dry matter than would have been anticipated on the basis of Wood's feeding standards. The actual consumption was 87, 80, 87 and 81 per cent of the predicted amounts for sheep 3, 4, 6 and 7 respectively.

During subperiod 3 of the grazing trial, however, the dry-matter consumption of the sheep (excepting the case of sheep 5) was in reasonable accord with Wood's standards, the mean daily amounts for sheep 3, 4, 6 and 7 being 106, 101, 102 and 94 per cent of the predicted values. Although these figures represent a fall in appetite compared with the results for sheep 1, 2, 8 and 9 on the superior herbage of the May grazing trial, it is nevertheless evident that even on grass of poorer quality and digestibility, the animals ate a larger ration, in terms of lb. dry matter, than they would have eaten if subsisting on a diet of hay, swedes and concentrates. Adopting the method of calculation already explained, it is seen that the extent of this increase of appetite was 0.81, 0.95, 0.82 and 0.59 lb. of dry matter per day for sheep 3, 4, 6 and 7 respectively.

During subperiod 4 (11-16 June), when the grass had undergone further deterioration in quality and digestibility, the appetites of the sheep showed a corresponding decline, the dry-matter consumption, with the exception of sheep 3, now being distinctly lower than the predicted amounts (100, 90, 92 and 90 per cent of the predicted values for sheep 3, 4, 6 and 7 respectively). Even under these conditions, however, the animals were still eating more dry matter per day than would have been expected from their appetites on the winter diet during March, the increases for sheep 3, 4, 6 and 7 being 0.57, 0.43, 0.27 and 0.37 lb. of dry matter per day respectively.

The results for sheep 5 have been ignored in the foregoing discussion. It was noted at the time that this animal appeared to be suffering from the effects of the hot, dry weather during the grazing trial, and this circumstance must be held responsible for its relatively poor appetite while on grass.

Pure-bred Suffolk wethers were used in the 1934 trials; in the trials of 1935 the wethers were by a Suffolk ram out of Cheviot  $\times$  Border Leicester ewes. A further distinguishing feature of the 1935 trials was the condition of the experimental pasture at the beginning of the experiment. As a consequence of the dry and abnormally cold weather during May (there was a night frost of exceptional severity on 17 May) the growth of grass was unusually late. The abundance of rich, green

herbage covering the plots at the beginning of the 1934 experiment gave way in the 1935 trial to a rather thin, slow-growing herbage of relatively poor colour and quality. It was felt that the palatability of the grass might not be so high as in a normal spring and that although the digestibility of the herbage might be satisfactory, this lack of high palatability might be reflected in the results of the appetite measurements. That this was the case is revealed by the figures in Table VII.

Table VII. *Appetites, in terms of lb. dry matter, of sheep on (a) pasture, and (b) a typical winter ration*

	Grazing trial (May to June 1935)				On typical winter ration* (Feb. 1935)		
	Mean daily consumption on grass		Mean live-weight of sheep (25 May to 9 June)	Appetite as predicted from Wood's feeding standards			
	Subperiod 1 (25 May to 1 June)	Subperiod 2 (2-9 June)			Mean daily consumption (9-23 Feb.)	Mean live-weight of sheep (9-23 Feb.)	Appetite as predicted from Wood's feeding standards
	lb. dry matter	lb. dry matter			lb. dry matter	lb.	lb. dry matter
Sheep 10	3.40	3.88	150	4.50	3.01	131	4.10
Sheep 11	4.58	4.09	140	4.30	3.17	125	3.96
Sheep 12	4.49	4.28	150	4.50	3.30†	125†	3.96
Sheep 14	4.85	4.43	155	4.60	3.50	129	4.05
Digestion coefficient of organic matter of grass	79.4	78.5					

\* Consisting of 300 g. flaked maize plus 150 g. decorticated ground nut cake (completely eaten in all cases) with chaffed meadow hay and thousand-head kale *ad lib.*

† Over period 22 Jan. to 8 Feb.

On the winter diet during February, sheep 11, 12 and 14 gave results very similar to those for the sheep in the 1934 trials, the animals consuming approximately 80, 83 and 86 per cent respectively of the amounts of dry matter predicted from Wood's standards. Sheep 10, however, was definitely a poorer feeder, its mean daily consumption of dry-matter on the winter ration being only 73 per cent of the predicted amount. The inferior appetite of this sheep was again evident during the grazing trial, its mean daily consumption of dry matter over the whole period (25 May to 9 June) being only 80 per cent of the amount predicted from the standards, compared with 108, 98 and 101 per cent respectively for sheep 11, 12 and 14.

It is clear from Table VII, however, that all the sheep, including even sheep 10, displayed a bigger appetite on grass than on the winter diet. Assuming that, at their live-weights during the grazing trial, they would have eaten the same percentage of the predicted amounts of dry-matter from the winter ration as they ate during the February trial, it can be shown that the improvement of appetite on grass amounted to 0.35,



1.20, 0.65 and 0.68 lb. of dry matter per day for sheep 10, 11, 12 and 14 respectively. The disparities between the appetites on spring pasture and on the winter diet are naturally not so striking as in the first grazing period of 1934, when the quality of the early grass was much superior to that available in 1935. As might be expected, the 1935 results display a closer harmony with those for subperiod 3 in 1934, when the quality of the grass had begun to deteriorate and the appetites of the sheep had also begun to display a corresponding decline.

#### SUMMARY

A technique for measuring the appetites of sheep on pasture is described.

Results are given to show that sheep consume a bigger ration, in terms of lb. dry matter, when on pasture than when subsisting out-of-doors on the winter type of diet composed of hay, swedes (or kale) and concentrates. This difference was noted even on grass that had deteriorated in quality, in one case as a result of hot, dry weather in early summer, and in a second case as a consequence of cold, dry weather in spring. With young, leafy pasturage at its best, however, the distinction is most marked.

In the March of 1934, to quote but one example, four pure-bred Suffolk wethers, of live-weight varying from 120 to 181 lb., were shown to consume, on a diet of concentrates (350 g. daily), swedes (*ad lib.*) and chaffed hay (*ad lib.*), only 80–88 per cent of the amounts of dry matter predicted on the basis of Wood's feeding standards. During the following May, when grazing leafy pasturage of very good quality (digestion coefficient of organic matter=81.1 per cent), the appetites of the same sheep (range of live-weight now from 137 to 201 lb.) were found to have undergone a striking stimulation, the mean daily consumption of dry matter now being from 104 to 117 per cent of the amounts predicted from the standards. This implied that the sheep were consuming 1.13, 1.37, 1.13 and 1.53 lb. respectively more dry matter per day than they would have eaten had the diet consisted of hay, concentrates and swedes. The increased appetite is to be attributed to the superior palatability of the young grass.

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- (3) WOOD & WOODMAN. *Bull. Minist. Agric.*, Lond. (1936), No. 48.
- (4) WOODMAN, BLUNT & STEWART. *J. agric. Sci.* (1926), **16**, 205.

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## (1) 1934 trials

	Subperiod 1 (10-17 May)					Subperiod 2 (18-24 May)				
	1	2	8	9		1	2	8	9	
Mean weight wet faeces per day (g.)	2433.0	2458.0	2425.0	3618.0		2643.0	2048.0	2807.0	3829.0	
Mean weight dry matter voided per day (g.)	464.2	463.3	492.5	615.8		526.5	540.7	514.2	630.6	
Mean weight ash voided per day (g.)	88.4	87.7	91.9	121.4		93.5	99.3	92.2	111.1	
Mean weight organic matter voided per day (g.)	375.8	375.6	400.6	494.4		433.0	441.4	422.0	519.5	
Organic matter voided from cotton cake per day (g.)	37.6	37.6	37.6	37.6		37.6	37.6	37.6	37.6	
Organic matter voided from grass per day (g.)	338.2	338.0	363.0	456.8		395.4	403.8	384.4	481.9	
Digestion coefficient of organic matter in grass (%)	81.7	81.7	81.7	81.7		80.4	80.4	80.4	80.4	
Mean weight grass organic matter consumed per day (g.)	1848.0	1847.0	1884.0	2496.0		2017.0	2060.0	1961.0	2459.0	
Mean weight grass dry matter consumed per day (lb.)	4.47	4.47	4.80	6.04		4.87	4.98	4.73	5.94	
Mean weight cake dry matter consumed per day (lb.)	0.19	0.19	0.19	0.19		0.19	0.19	0.19	0.19	
Mean total dry matter consumed per day (lb.)	4.66	4.66	4.99	6.23		5.06	5.17	4.92	6.13	

	Subperiod 3 (5-10 June)					Subperiod 4 (11-16 June)				
	3	4	5	6	7	3	4	5	6	7
Mean weight wet faeces per day (g.)	2901.0	3836.0	2593.0	4227.0	3106.0	2789.0	3230.0	2674.0	3859.0	2985.0
Mean weight dry matter voided per day (g.)	608.3	619.1	552.8	746.9	576.2	609.7	588.2	583.5	720.1	590.4
Mean weight ash voided per day (g.)	100.2	103.6	93.2	117.6	93.6	98.7	102.5	96.6	115.9	104.8
Mean weight organic matter voided per day (g.)	508.1	515.5	459.6	629.3	482.6	511.0	485.7	486.9	604.2	485.6
Organic matter voided from cotton cake per day (g.)	37.6	37.6	37.6	37.6	37.6	37.6	37.6	37.6	37.6	37.6
Organic matter voided from grass per day (g.)	470.5	477.9	422.0	591.7	445.0	473.4	448.1	449.3	566.6	448.0
Digestion coefficient of organic matter in grass (%)	73.7	73.7	73.7	73.7	73.7	71.8	71.8	71.8	71.8	71.8
Mean weight grass organic matter consumed per day (g.)	1789.0	1817.0	1605.0	2250.0	1692.0	1679.0	1589.0	1593.0	2009.0	1589.0
Mean weight grass dry matter consumed per day (lb.)	4.31	4.37	3.86	5.42	4.07	4.07	3.85	3.86	4.87	3.85
Mean weight cake dry matter consumed per day (lb.)	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Mean total dry matter consumed per day (lb.)	4.50	4.56	4.05	5.61	4.26	4.26	4.04	4.05	5.06	4.04

## (2) 1935 trials

	Subperiod 1 (25 May to 1 June)					Subperiod 2 (2-9 June)				
	10	11	12	14		10	11	12	14	
Mean weight wet faeces per day (g.)	1946.0	2542.0	2107.0	2591.0		2537.0	3314.0	2724.0	2805.0	
Mean weight dry matter voided per day (g.)	363.2	475.0	467.4	503.0		429.1	517.6	470.2	489.8	
Mean weight ash voided per day (g.)	61.9	76.8	76.0	82.5		66.9	83.6	73.1	78.7	
Mean weight organic matter voided per day (g.)	301.3	398.2	391.4	420.5		362.2	434.0	397.1	411.1	
Organic matter voided from cotton cake per day (g.)	37.6	37.6	37.6	37.6		37.6	37.6	37.6	37.6	
Organic matter voided from grass per day (g.)	263.7	360.6	353.8	382.9		324.6	396.4	359.5	373.5	
Digestion coefficient of organic matter in grass (%)	79.4	79.4	79.4	79.4		78.5	78.5	78.5	78.5	
Mean weight grass organic matter consumed per day (g.)	1280.0	1750.0	1717.0	1859.0		1510.0	1844.0	1672.0	1735.0	
Mean weight grass dry matter consumed per day (lb.)	3.21	4.39	4.30	4.66		3.69	4.50	4.09	4.24	
Mean weight cake dry matter consumed per day (lb.)	0.19	0.19	0.19	0.19		0.19	0.19	0.19	0.19	
Mean total dry matter consumed per day (lb.)	3.40	4.58	4.49	4.85		3.88	4.69	4.28	4.43	

Note: The care of the experimental animals throughout the trials was in the hands of Messrs V. Thurlbourn and C. Bendall.

# THE TIME OF CUTTING HAY, AND THE LOSSES ENTAILED DURING HAYMAKING

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THE most general method of conservation of fodder on the farm is that of haymaking. Unfortunately, many factors combine to make the hay crop one of the most variable on the farm as regards composition and feeding value.

The cost of production of hay can be reduced by allowing the crop to grow to maturity, when high yields are obtained, but there will have been a definite falling off in nutritive value during the latter stages of growth. There is a general tendency in this country to produce quantity of fodder, rather than quality, but the most economic return will be obtained when the yields of starch equivalent and protein equivalent are greatest.

To obtain this maximum yield of nutritive material, it is most important to know at which stage of growth the crop should be cut, but it is also important to know what losses are likely to arise during making. Local climatic conditions may be habitually unsuitable for haymaking when the crop is at an ideal stage, and would impose such losses as to make it profitable to delay cutting.

No information is available apparently as to the losses which occur during haymaking in the British Isles, but much work has been done on the Continent. Wiegner<sup>(1)</sup> summarizes the losses as follows:

Table I. *Losses due to haymaking, compared with the  
nutrients in the fresh crop*

	Dry matter %	Digestible dry matter %	Starch equivalent %
Respiration	Up to 10	5-15	5-15
Mechanical losses	5-10	5-10	5-10
Fermentation in the stack	5-10	5-10	5-10
Loss due to metabolic processes in animal	—	—	10-15
Total	10-30	15-35	25-50

The figures given refer to average conditions, but when the hay is subjected in the field to the leaching action of rain, the losses may be greatly increased. Crasemann & Steiner<sup>(2)</sup> have shown that the losses of starch equivalent and digestible true protein increase with the amount of rain falling on the crop in the field, and report, in an extreme case, a loss of 62 per cent of the starch equivalent of the fresh crop and 45 per cent of the digestible true protein.

It was decided to investigate the yield of nutrients from hay cut at two different stages of growth, and to determine the losses occurring during the making and stacking. The first type of hay was cut at about the normal time for haymaking in this district and when the majority of the grasses had flowered and the earlier grasses had shed their seed. The second type was cut some 3-4 weeks earlier, when the majority of the grasses were just coming into flower. The hays will be referred to as ordinary and early hay.

In estimating the yields from the early hay, the growth which took place on the early hay plots between the times of cutting the early and late hay had to be included, since this herbage would have been available for grazing under practical conditions.

The work was commenced in 1930 and continued until 1935. It will be convenient to consider the composition, nutrient content and yields of the hay year by year, and then to deal finally with the losses involved.

### 1930

The experiment in 1930 was of a preliminary nature, being part of another investigation dealing with the conservation of grassland herbage by various methods.

Only early hay was considered, and no yield or loss data were obtained. The composition and nutrient content of the material are given, however, to show the feeding value of the hay when cut at such an early stage. Also an account of the weather conditions is of value, since the observations over a number of years might indicate whether the conditions were generally suitable or otherwise for haymaking at the time chosen for cutting early hay.

The grass was cut on 16 May, which was about 5 weeks before the normal date of cutting in 1930. Bad haymaking conditions prevailed, and the hay was not finally stacked until 4 June. Rain fell on 12 days whilst the hay was in the field, and to assist making, the hay was built into small cocks, but nevertheless a good deal of leaching of soluble constituents must have taken place.

## 226 *Time of Cutting Hay, and Losses during Haymaking*

The hay was finally built into a small stack in which fermentation seemed quite normal, and no undue heating of the material was evident from the appearance of the final product.

The digestibility of the hay was determined, using sheep as the experimental animals. In Table II the composition, digestibility, and digestible nutrient content of the early hay are given, together with the composition of the grass from which the hay was made, and the digestible nutrients in good meadow hay as quoted by Kellner.

Table II. *Composition, digestibility and digestible nutrient content of early hay, composition of grass from which early hay was made, and nutrient content of good meadow hay. 1930*

	Grass Composition*	Early hay			Good meadow hay (Kellner) Digestible nutrients*
		Composition*	Digestibility %	Digestible nutrients*	
Ether extract	2.08	1.82	36.2	0.66	1.2
Fibre	22.84	26.37	82.6	21.78	17.5
Crude protein	16.20	16.25	64.9	10.55	6.3
Ash	12.63	10.39	—	—	—
N-free extractives	46.25	45.17	71.9	32.48	30.0
Organic matter	87.37	89.61	73.3	—	—
True protein	11.37	13.53	61.1	8.27	4.4
Calcium (CaO)	0.88	0.85	—	—	—
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.81	0.79	—	—	—
Starch equivalent	—	—	—	47.9	36.2
Protein equivalent	—	—	—	9.41	5.35
Dry matter in fresh material	20.2	86.5	70.6	—	—

\* Stated as percentages of the dry matter.

Considering the figures for the composition of the early hay and the grass, it is seen that the fibre is appreciably higher in the hay, indicating a loss of some of the other constituents. It would appear that all the other constituents suffered losses, with the possible exception of the true protein, since if the loss had fallen on only one constituent, the remainder would have shown increased values as compared with the original grass.

The digestibility of the hay was satisfactory, and the digestible nutrient content compares favourably with that of good meadow hay, showing appreciably higher values for starch equivalent and protein equivalent. The most noticeable point, as would be expected, was the high protein content of the early hay.

The experiment has shown that hay of high quality can be produced by early cutting, even under fairly adverse weather conditions.

1931

It was impossible to obtain a really suitable site for the experiment in 1931. Consequently, no replication of the plots was obtained, and the determination of the losses during haymaking had to be abandoned.

The two hay areas selected were situated together in a large field of newly sown grass in its second harvest year. The field was even as regards productivity, as far as could be judged by eye, but no estimates of yields of fresh material were made at the commencement of the experiment. For the purpose of this experiment it has to be assumed that the areas selected were uniform in character.

The early hay was cut on 26 May, when the grass was 10–12 in. long, the size of the area cut being 2 acres. Weather conditions were again poor, and 0.75 in. of rain fell on the crop while it was lying in the field. The hay was finally led to the stack on 1 June.

The rain had a favourable effect on the growth of the grass on the ordinary hay plot, which, when cut on 15 June, was about 2–2½ ft. high. One acre was cut, and the weather conditions at this time were more favourable for haymaking, only 0.33 in. of rain falling before the hay was stacked on 21 June.

At the same time that the ordinary hay was mown, a large strip was cut on the area previously cut for early hay. The weight of produce obtained was 707 lb. of dry matter per acre, and in calculating the yield of nutrients from the two treatments, this has to be included in the early hay treatment.

#### *Composition and digestibility*

The composition and digestibility of the hays and the aftermath are given in Table III.

The early hay shows a higher protein content and a lower fibre content than the ordinary hay, but these differences are not as marked as was expected from the appearance and stage of growth of the two crops. The composition and digestibility of the aftermath were poor owing to unavoidable contamination with the stubble from the first crop.

There was surprisingly little difference in digestibility between the early and ordinary hays, although the early hay was generally slightly superior.

#### *Digestible nutrients.*

The digestible nutrient content of the hays and aftermath, together with Kellner's values for good meadow hay, are given in Table IV.

## 228 *Time of Cutting Hay, and Losses during Haymaking*

Table III. *Composition and digestibility of early hay, ordinary hay, and the aftermath from the early hay area. 1931*

	Early hay		Ordinary hay		Aftermath	
	Composi- tion*	Digesti- bility %	Composi- tion*	Digesti- bility %	Composi- tion*	Digesti- bility %
Ether extract	1.54	32.0	1.21	37.2	2.48	51.8
Crude fibre	34.71	78.7	37.29	70.4	28.78	76.9
Crude protein	10.40	48.8	7.23	46.7	12.47	51.8
Ash	9.04	—	8.59	—	11.68	—
N-free extractives	44.31	64.5	45.68	63.7	44.59	74.0
Organic matter	90.96	68.0	91.41	62.2	88.32	71.3
True protein	8.60	41.8	6.54	43.6	11.08	50.7
Calcium (CaO)	0.65	—	0.75	—	0.95	—
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.67	—	0.77	—	0.81	—
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0.83	—	0.90	—	0.89	—
Dry matter in fresh material	82.1	65.2	83.8	61.7	—	67.1

\* Stated as percentages of the dry matter.

Table IV. *Digestible nutrient content of early hay, ordinary hay, aftermath from the early hay area, and good meadow hay. 1931. (Stated as percentages of the dry matter)*

	Early hay	Ordinary hay	Aftermath	Good meadow hay (Kellner)
Digestible organic matter	61.8	56.9	62.9	—
Digestible ether extract	0.5	0.5	1.3	1.2
Digestible fibre	27.3	26.3	22.1	17.5
Digestible N-free extractives	28.6	29.1	33.0	30.0
Digestible crude protein	5.1	3.4	6.5	6.3
Digestible true protein	3.6	2.9	5.6	4.4
Starch equivalent	40.1	37.3	53.0	36.2
Protein equivalent	4.3	3.1	6.0	5.4

The early and ordinary hays do not compare favourably with the good meadow hay, for although they contain about the same starch-equivalent value, their protein contents are definitely lower. This was unexpected in the case of the early hay, since this material was cut when only 10–12 in. long, and presumably contained a fairly high content of crude protein. It must be assumed that, during the making, large losses of crude protein arose through leaching by rain-water. That large losses of some constituent did occur is supported by the fibre content of the hay, the value being very high for such relatively immature herbage.

The starch equivalent of the aftermath is higher than the hays, this being mainly due to the lower fibre correction factor used in the calculation. The protein content, however, was little better than that of good meadow hay.

*Yield data*

The early hay when removed from the stack gave a yield of dry matter corresponding to 1357 lb. per acre, and the aftermath to be added to this amounted to 707 lb. The ordinary hay produced 3237 lb. per acre.

From the yields of dry-matter and nutrient contents of the material, the production of nutrients per acre has been calculated and is given in Table V.

Table V. *Yield of dry matter, crude protein and nutrients in early and ordinary hay. 1931. (Stated as lb. per acre)*

	Early hay			Ordinary hay	Relative yields, ordinary hay = 100
	1st cut	Aftermath	Total		
Dry matter	1357	707	2064	3237	64
Crude protein	141	88	229	234	98
Digestible true protein	48.7	39.7	88.4	92.3	96
Starch equivalent	544	375	919	1207	76
Protein equivalent	59	45	104	101	103

The yields of crude protein, digestible true protein and protein equivalent were approximately the same in the two treatments, but while the ordinary hay produced 3237 lb. of dry matter per acre, or 34 cwt. of hay, and 1207 lb. of starch equivalent, the early hay area produced only about two-thirds and three-quarters of these amounts respectively.

Under the conditions obtaining in 1931, it is evident that the early cutting of the hay resulted in a definite loss of energy-producing material as compared with the hay cut at the normal time.

## 1932

In 1932 an experiment was designed to investigate a number of methods of conservation. Six methods were tried—early hay and ordinary hay, ensilage made in three ways, and artificial drying.

In order to ensure that the material used in all the treatments was uniform, a random block lay-out with five replications was adopted, each plot being 0.3 of an acre in extent.

All the treatments, with the exception of the ordinary hay, were cut on 13 June under good weather conditions. The weather remained fine and sunny for 4 days, and no rain fell. The hay was made quickly and well, and was carted from the field on 16 June.

The produce from the silage and artificially dried grass plots was removed from the field, one plot at a time, weighed and sampled for determination of dry-matter content.



## 230 *Time of Cutting Hay, and Losses during Haymaking*

It was not thought desirable to weigh the whole of the fresh herbage on the early hay plots, since this would entail disturbing all the swathes. It was therefore decided to take the average yield of fresh grass obtained from the other four treatments as a measure of the fresh-grass yield on the early hay plots, this value being required for subsequent determination of losses.

The hay was made on the plots in the usual way with farm implements, the final raking and collection, however, being done by hand. As the hay was led from the field it was weighed, each plot separately, and sampled.

The hay was built into a small stack between two layers of other hay, from which it was separated by wire netting.

The ordinary hay on the farm was cut at a late date in this year, and the ordinary hay plots were not cut until 11 July, 4 weeks after the cutting of the early hay. A thunderstorm followed the cutting, and 0.23 in. of rain fell on the freshly cut crop. The weather was generally dull, and slight showers of rain delayed stacking until 19 July.

The yield of fresh grass per plot was determined by cutting three swathes through each plot with the farm mowing-machine, weighing and sampling the produce for dry-matter determination.

When the hay was made, it was weighed and stacked in a similar way to the early hay.

At the same time as the ordinary hay plots were cut, the growth on the early hay plots was measured. Since this would normally have been grazed, and the animal would graze closer than an ordinary mower would cut, the plots were sampled by means of a lawn-mower. Three cuts were taken at random along the greatest length of each plot. The area cut was measured, and the weight of fresh grass and its dry-matter content determined.

The digestibility was determined on the fresh grass at the time of cutting for hay, the hay leaving the field, and the hay when cut out of the stack. The digestibility of the fresh-grass aftermath was also measured.

### *Composition*

#### *Early hay.*

The composition, digestibility, and digestible nutrient content of the hay at the various stages are given in Table VI.

The composition and digestibility of the hay when carted from the field is very similar to that of the fresh grass, although the true protein of the hay shows slightly depressed digestibility. The starch equivalent is lower in the hay, but this is due chiefly to the differences in the correction

by virtue of the higher fibre content—the correction for “value” (or Wertigkeit) of Kellner.

Table VI. *Composition, digestibility and digestible nutrients in the fresh grass and early hay. 1932*

	Fresh grass			Early hay					
				Ex field			Ex stack		
	C.*	D. %	D.N.*	C.*	D. %	D.N.*	C.*	D. %	D.N.*
Ether extract	1.89	57.7	1.09	1.86	41.0	0.76	1.97	44.0	0.87
Fibre	24.63	77.5	19.09	23.95	76.3	18.27	26.85	77.9	20.91
Crude protein	11.77	68.9	8.11	12.20	66.5	8.11	12.94	48.5	6.27
Ash	7.59	—	—	7.73	—	—	8.22	—	—
N-free extractives	54.12	81.9	44.32	54.26	79.9	43.35	50.02	70.4	35.21
Organic matter	92.41	78.6	72.63	92.27	76.1	70.22	91.78	69.0	63.32
True protein	10.40	67.1	6.98	9.89	62.6	6.19	10.44	48.3	5.04
Calcium (CaO)	0.67	—	—	0.58	—	—	0.72	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.70	—	—	0.70	—	—	0.77	—	—
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0.88	—	—	0.81	—	—	0.81	—	—
Starch equivalent	—	—	63.7	—	—	55.0	—	—	47.0
Protein equivalent	—	—	7.55	—	—	7.15	—	—	5.66
Dry matter in fresh material	24.8	77.3	—	74.7	74.2	—	83.4	66.8	—

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

The fermentation in the stack has had a more marked effect on the composition, the N-free extractives falling and the crude protein and fibre rising. The chief difference, however, shows in the lowered digestibility of the protein, which is reflected in the protein-equivalent value. The starch equivalent has also fallen below the level found in the hay as it left the field.

#### *Ordinary hay.*

The data for ordinary hay are given in Table VII.

The fresh grass shows signs of the advance of maturity as compared with the grass at the early hay stage (Table VI). The most noticeable feature is a fall in the amount of crude protein and a slight increase in fibre. In addition, there has been a fall in the digestibility of the constituents, which is again most marked in the case of the protein and fibre. As a result, the starch-equivalent and protein-equivalent values have fallen considerably.

The process of haymaking in the field resulted in a loss which showed up most clearly in the protein content. As the hay was not subjected to any great leaching effect by rain, it is probable that this fall in protein

## 232 *Time of Cutting Hay, and Losses during Haymaking*

content was caused by mechanical loss of the more leafy portion of the herbage. The digestibility of the protein was also seriously depressed, and the N-free extractives also showed lowered digestibility as compared with the fresh grass.

Table VII. *Composition, digestibility and digestible nutrients in the fresh grass and ordinary hay. 1932*

	Fresh grass			Ordinary hay					
				Ex field			Ex stack		
	C.*	D. %	D.N.*	C.*	D. %	D.N.*	C.*	D. %	D.N.*
Ether extract	2.04	52.7	1.07	1.13	15.4	0.17	1.39	11.0	0.15
Fibre	26.50	61.8	16.38	30.58	64.0	19.57	30.30	65.8	19.94
Crude protein	8.99	63.4	5.70	6.61	35.9	2.37	8.31	50.3	4.18
Ash	6.90	—	—	6.26	—	—	7.20	—	—
N-free extractives	55.57	76.8	42.68	55.42	66.3	36.74	52.80	66.2	34.95
Organic matter	93.10	70.7	65.82	93.74	62.1	58.21	92.80	63.8	59.21
True protein	7.73	59.4	4.59	5.32	27.6	1.47	7.10	47.4	3.36
Calcium (CaO)	0.76	—	—	0.64	—	—	0.82	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.48	—	—	0.52	—	—	0.50	—	—
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0.86	—	—	0.80	—	—	0.98	—	—
Starch equivalent	—	—	50.0	—	—	40.3	—	—	40.8
Protein equivalent	—	—	5.15	—	—	1.92	—	—	3.77
Dry matter in fresh material	27.9	69.1	—	74.0	60.7	—	83.8	62.7	—

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

The fermentation in the stack has not greatly affected the composition of the hay, although the protein shows an increased value.

The chief point of interest is the apparent building up of "true" protein (copper-precipitable nitrogen  $\times 6.25$ ) as judged from the ratio of "true" to crude protein, and the fermentation has had a beneficial effect on the digestibility of the protein, though this is still below the level of the fresh grass. The other constituents have not been appreciably altered, and the starch equivalent of the hay from the stack is similar to that of the hay as it left the field, though in protein equivalent the former shows a decided increase.

### *Aftermath.*

The aftermath is the material which was cut from the early hay plots at the time of cutting the ordinary hay. The composition and digestibility of the material are given in Table VIII.

The material is of similar nutritive value to the herbage cut at the early hay stage, but with a slightly higher protein-equivalent value.

Table VIII. *Composition, digestibility and digestible nutrients in aftermath cut 1932*

	Composition*	Digestibility %	Digestible nutrients*
Ether extract	2.36	51.1	1.21
Fibre	24.22	76.3	18.48
Crude protein	12.35	71.0	8.77
Ash	9.48	—	—
N-free extractives	51.59	78.2	40.34
Organic matter	90.52	73.2	66.26
True protein	10.86	69.2	7.51
Calcium (CaO)	0.96	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.64	—	—
Ratio True protein Crude protein	0.88	—	—
Starch equivalent	—	—	60.0
Protein equivalent	—	—	8.14
Dry matter	24.9	73.7	—

\* Stated as percentages of the dry matter.

The values are lowered to some extent by the presence of the stubble from the early cut. The grazing animal would leave this dead material, but the lawn-mower cannot avoid it. Under grazing conditions a lesser bulk of more nutritive material would have been obtained, but the method adopted is the only practicable way of measuring the growth.

#### *Yield data*

Owing to a clerical error during the weighing out of the early hay from the stack, a number of the bales into which the hay was cut were included twice, and so it is impossible to give the weight of early hay removed from the stack. No obvious over-heating had occurred in the stack, and the fermentation appeared quite normal; and for the purpose of calculating the yield data, an assumption will be made that the loss of dry matter in the stack amounted to 5 per cent.

It is not intended to give the yield data for the individual plots in this paper, and the figures quoted are average values calculated to yield per acre.

The yields of dry matter and nutrients obtained are given in Table IX.

The yields were definitely less than in 1931, the ordinary hay producing only 2217 lb. dry matter, or approximately 23 cwt. of fresh hay per acre. It will be seen later that the losses sustained by the crop in the field contributed largely to the low return of the ordinary hay.

The early hay, while producing only about three-quarters of the weight of dry matter of the ordinary hay, gave the same yield of starch equivalent, and approximately one-third more protein equivalent and

## 234 *Time of Cutting Hay, and Losses during Haymaking*

digestible true protein. These results were obtained in a year when the weather conditions were good at the time of making the early hay, and poor when the ordinary hay was made.

Table IX. *Yields of dry matter, crude protein and nutrients in early and ordinary hay. 1932. (Stated as lb. per acre)*

	Early hay			Ordinary hay	Relative yields, ordinary hay = 100
	1st cut	Aftermath	Total		
Dry matter	1067	623	1690	2217	76
Crude protein	138	77	215	184	117
Digestible true protein	53.8	46.8	100.6	74.5	135
Starch equivalent	502	374	876	905	97
Protein equivalent	60.4	50.7	111.1	83.6	133

1933

The experiment in 1933 was in a field of permanent grass of good quality.

On 17 and 18 May the bulk of the grass on the field was cut for silage. During the cutting, six blocks of grass were left uncut, and these were distributed at random over the field. Each block was isolated by cutting round and round until a suitable area was left in the centre.

The grass for early hay was cut on 19 May. From these blocks, two swathe cuts 41 in. wide and approximately 178 ft. long were made on each block. The weight of fresh grass in each swathe was measured, the material sampled for dry-matter determination, and then laid out on the area surrounding the blocks which had been cut previously. The length and width of the swathe cuts were measured to enable the yield to be referred to the acreage.

The weather at this time was fine, but heavy rain fell 3 days after cutting, and the hay was not gathered until 26 May.

The yield of hay from each swathe was determined, and the hay sampled separately from each swathe. A composite sample was made up for the determination of digestibility. The hay was wrapped up separately, according to swathes, in sacks of open-mesh hessian, and the twelve samples were built into a large stack of hay in a Dutch barn, being placed at different points throughout the stack. In winter, the samples were removed from the stack, weighed and sampled separately, and a composite sample made up for the determination of digestibility.

On 27 June the ordinary hay was cut, this being the time when the hay crop was being cut locally. The method adopted was identical with that for early hay, two swathes being cut on each of the six blocks.

The hay made well in fine sunny weather, no rain falling, and was stacked on 30 June.

On the same day as the grass was cut for ordinary hay, an aftermath cut was taken on each of the swathes previously cut for early hay, a "Cut-Rough" lawn-mower being used for the purpose. The yield, dry-matter content and digestibility of the fresh grass, and the area cut, were all measured.

### Composition

#### Early hay.

The composition and digestibility of the fresh grass, the hay ex field and the hay ex stack were all measured, and the figures are summarized below, together with the digestible nutrients in the materials.

Table X. *Composition, digestibility and digestible nutrients in the fresh grass and early hay. 1933*

	Fresh grass			Early hay					
				Ex field			Ex stack		
	C.*	D. %	D.N.*	C.*	D. %	D.N.*	C.*	D. %	D.N.*
Ether extract	2.63	52.3	1.37	2.02	49.1	0.99	2.30	50.9	1.17
Fibre	26.33	82.8	21.80	27.41	76.0	20.83	28.19	82.8	23.33
Crude protein	13.89	74.0	10.28	13.74	62.7	8.62	14.19	64.0	9.08
Ash	8.77	—	—	7.68	—	—	7.99	—	—
N-free extractives	48.38	78.2	37.85	49.15	70.5	34.65	47.33	69.6	32.94
Organic matter	91.23	77.8	70.96	92.32	70.4	64.98	92.01	72.3	66.57
True protein	12.44	70.2	8.74	11.68	61.1	7.13	12.14	65.8	7.98
Calcium (CaO)	0.57	—	—	0.50	—	—	0.58	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.93	—	—	0.80	—	—	0.87	—	—
Ratio True protein Crude protein	0.90	—	—	0.85	—	—	0.86	—	—
Starch equivalent	—	—	63.7	—	—	48.2	—	—	49.6
Protein equivalent	—	—	9.51	—	—	7.88	—	—	8.53
Dry matter in fresh material	22.7	74.4	—	79.5	67.7	—	87.2	70.0	—

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

There is little difference in composition between the fresh grass and the hay ex field or ex stack.

With the exception of the ether extract and fibre, there was a general diminution in digestibility of the hay. The starch-equivalent value of the hay is lower than that of the fresh grass, chiefly as a result of the application of the higher factor in correcting the gross energy of the hay for its fibre content.

The protein equivalent is very satisfactory, and the hay was of high

## 236 *Time of Cutting Hay, and Losses during Haymaking*

nutritive value, and compares very favourably with that of the ordinary hay (Table XI). The digestible nutrients appear to have increased as a result of the fermentation in the stack.

### *Ordinary hay.*

The figures for the composition and digestibility of the fresh grass, the hay as it left the field, and the hay from the stack are given in Table XI.

Table XI. *Composition, digestibility and digestible nutrients in the fresh grass and ordinary hay. 1933*

	Fresh grass			Ordinary hay					
				Ex field			Ex stack		
	C.*	D. %	D.N.*	C.*	D. %	D.N.*	C.*	D. %	D.N.*
Ether extract	2.38	60.2	1.43	2.08	52.1	1.08	2.15	45.5	0.98
Fibre	30.57	64.5	19.71	30.29	64.1	19.40	29.36	62.7	18.40
Crude protein	7.55	54.3	4.10	7.59	46.8	3.55	7.88	42.7	3.36
Ash	6.76	—	—	7.10	—	—	7.28	—	—
N-free extractives	52.74	68.1	35.90	52.94	66.4	35.13	53.33	62.9	33.54
Organic matter	93.24	65.5	61.07	92.90	63.8	59.27	92.72	60.7	56.28
True protein	6.44	51.7	3.33	6.87	42.0	2.89	7.12	42.8	3.05
Calcium (CaO)	0.51	—	—	0.54	—	—	0.67	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.60	—	—	0.61	—	—	0.58	—	—
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0.85	—	—	0.90	—	—	0.90	—	—
Starch equivalent	—	—	49.2	—	—	41.7	—	—	39.9
Protein equivalent	—	—	3.72	—	—	3.22	—	—	3.21
Dry matter in fresh material	32.7	63.9	—	79.2	61.8	—	88.2	59.4	—

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

There has been little change in composition, probably owing to the good conditions under which the hay was made.

The digestibility of the protein fell markedly during haymaking, as did that of the ether extract, whilst the N-free extractives showed some diminution.

The starch-equivalent value is lower than that of the early hay (Table X), but a most marked difference is seen in the protein-equivalent value, the value for the ordinary hay being under half that of the early hay.

### *Aftermath.*

The data relating to the aftermath are given in Table XII.

Table XII. *Composition, digestibility and digestible nutrients in aftermath cut 1933*

	Composition*	Digestibility %	Digestible nutrients*
Ether extract	3.11	68.6	2.13
Fibre	22.26	82.5	18.36
Crude protein	14.71	81.0	11.91
Ash	10.69	—	—
N-free extractives	49.23	81.8	40.27
Organic matter	89.31	81.4	72.70
True protein	13.33	77.8	10.37
Calcium (CaO)	0.96	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.89	—	—
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0.91	—	—
Starch equivalent	—	—	65.22
Protein equivalent	—	—	11.14
Dry matter	27.6	80.0	—

\* Stated as percentages of the dry matter.

The aftermath contained a lower fibre content and a slightly higher crude-protein content than the grass cut at the early hay stage. The digestibility of the aftermath was greater than that of the early hay grass and, as a result, the starch-equivalent value was slightly higher and the protein equivalent considerably higher in the aftermath.

#### *Yield data*

For the sake of brevity, the individual plot yields are not given here, only the average yields from all the twelve plots. An examination of the yields of dry matter from the individual plots when cut for hay and aftermath, shows that the total area used for the experiment was fairly even as regards productivity, this being truer at the time of cutting the early hay than at the later period. The standard error for the blocks gave the following values, the figures being calculated on the basis of lb. dry matter per acre:

	Mean yield	Standard error of means*
Grass for early hay + aftermath	3254	51
Grass for ordinary hay	5328	373

6 degrees of freedom.

The yields of dry matter and nutrients are given in Table XIII.

High yields were obtained in 1933, the production of ordinary hay being approximately 46 cwt. per acre, and the production of early hay being greater than that of the ordinary hay in 1932.



## 238 *Time of Cutting Hay, and Losses during Haymaking*

Table XIII. *Yields of dry matter, crude protein and nutrients in early and ordinary hay. 1933. (Stated as lb. per acre)*

	Early hay			Ordinary hay	Relative yields, ordinary hay = 100
	1st cut	Aftermath	Total		
Dry matter	2107	586	2693	4403	61
Crude protein	299	86	385	347	111
Digestible true protein	168	61	229	134	171
Starch equivalent	1045	382	1427	1757	81
Protein equivalent	180	65	245	141	174

The early hay yielded definitely less dry matter and starch equivalent than the ordinary hay, but more digestible true protein and protein equivalent. In actual weights per acre the ordinary hay gave 330 lb. more starch equivalent and 104 lb. less protein equivalent than the early hay.

These results were obtained in a favourable season when haymaking conditions were good, particularly so when the ordinary hay was made.

### 1934

The site of the experiment was the same as in 1933, and the design and lay-out were similar. The size of the plots was increased, however, and the replications reduced to five.

The bulk of the grass in the field was cut for silage, but five blocks were left uncut, distributed at random over the field.

The early hay plots were allocated at random within each block and were cut on 25 May. The areas of the plots, approximately one-twentieth of an acre, were measured, and the produce weighed and sampled in the field. The grass was then spread over the plot and allowed to dry. It was turned once, and was ready for stacking on 29 May. The weather conditions were very good at this time; no rain fell, and the temperatures were moderate. The hay from each area was weighed and sampled as it left the field, and a composite sample was made up for the determination of digestibility. The hay from each plot was wrapped up separately in open-mesh hessian, and placed at different points in a small stack. This stack was opened on 29 June, when the samples were again weighed and sampled. The hay was still warm, probably owing to the fresh nature of the accompanying hay, but it was not convenient to leave the material for a greater length of time.

The ordinary hay was cut on 15 June, and the procedure in this field was as for the early hay. The hay was gathered on 18 June, the weather being exceptionally fine; no rain fell, and temperatures were high. The

hay samples, wrapped in hessian, were built into a large stack in a Dutch barn, and remained there until 18 September.

After 25 May, when the early hay was cut, droughty weather conditions prevailed, and no aftermath had been produced on the early hay plots by the time the ordinary hay was cut.

### Composition

#### Early hay

The composition and digestibility of the fresh grass and hay are given in Table XIV, together with their digestible nutrient contents.

Table XIV. *Composition, digestibility and digestible nutrients in the fresh grass at the early hay stage, early hay ex field and early hay ex stack. 1934*

	Early hay								
	Fresh grass			Ex field			Ex stack		
	C.*	D. %	D.N.*	C.*	D. %	D.N.*	C.*	D. %	D.N.*
Ether extract	2.61	68.5	1.79	2.10	47.3	0.99	2.71	56.6	1.53
Crude fibre	24.76	75.0	18.57	25.12	74.4	18.69	26.36	74.4	19.61
Crude protein	13.15	68.1	8.96	13.09	67.8	8.88	14.12	65.2	9.21
Ash	8.40	—	—	8.33	—	—	9.31	—	—
N-free extractives	51.08	75.8	38.72	51.36	73.7	37.85	47.50	76.9	36.53
Organic matter	91.60	74.1	67.88	91.67	72.3	66.28	90.69	73.8	66.93
True protein	11.25	66.3	7.46	11.02	65.1	7.17	11.82	62.7	7.41
Calcium (CaO)	0.53	—	—	0.56	—	—	0.58	—	—
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.73	—	—	0.74	—	—	0.77	—	—
Ratio True protein	0.86	—	—	0.84	—	—	0.84	—	—
Crude protein	—	—	—	—	—	—	—	—	—
Starch equivalent	—	—	59.3	—	—	50.6	—	—	50.7
Protein equivalent	—	—	8.21	—	—	8.03	—	—	8.31
Dry matter in fresh material	24.9	72.5	—	79.0	70.5	—	83.9	72.5	—

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

The differences in composition between the grass and hays are slight, although the hay from the stack shows slightly higher values for crude fibre and crude protein, and a lower value for N-free extractives.

The differences in digestibility are also slight, the fresh grass on the whole giving higher values.

The protein is very similar in all three cases, and the starch equivalents of the hays, while being lower than the fresh grass, are of a high order.

#### Ordinary hay.

The composition and digestibility data are given in Table XV.

## 240 *Time of Cutting Hay, and Losses during Haymaking*

Table XV. *Composition, digestibility and digestible nutrients in fresh grass at the ordinary hay stage, ordinary hay ex field and ordinary hay ex stack. 1934*

	Ordinary hay								
	Fresh grass			Ex field			Ex stack		
	C.*	D.	D.N.*	C.*	D.	D.N.*	C.*	D.	D.N.*
Ether extract	2.80	57.2	1.60	2.01	45.9	0.92	2.60	50.0	1.30
Crude fibre	26.80	69.6	18.65	27.41	66.6	18.26	26.48	71.6	18.96
Crude protein	9.65	65.2	6.29	9.84	64.4	6.34	10.06	56.8	5.71
Ash	7.31	—	—	7.24	—	—	7.88	—	—
N-free extractives	53.44	74.6	39.87	53.50	74.2	39.70	52.98	70.6	37.40
Organic matter	92.69	71.7	66.46	92.76	70.2	65.12	92.12	68.7	63.29
True protein	7.90	59.5	4.70	7.98	58.4	4.66	8.26	53.6	4.43
Calcium (CaO)	0.51	—	—	0.52	—	—	0.61	—	—
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.50	—	—	0.51	—	—	0.52	—	—
Ratio True protein Crude protein	0.82	—	—	0.81	—	—	0.82	—	—
Starch equivalent	—	—	55.4	—	—	48.2	—	—	47.6
Protein equivalent	—	—	5.50	—	—	5.50	—	—	5.07
Dry matter in fresh material	31.4	70.8	—	90.1	69.1	—	88.1	67.4	—

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

The changes in composition arising during haymaking and in the stack were negligible.

The digestibility of the protein was lowered in the stack and the other constituents, with the exception of the fibre, also show slightly lower values.

The starch-equivalent values of the hays are high, and are close to those given by the early hay, but the protein-equivalent values are much lower.

### *Yield data*

A statistical examination was made of the yields of dry matter and crude protein in the early and ordinary hay at each stage of the experiment. It was found that no significant difference was evident between the two hays as regards yield of dry matter from the field and from the stack. This result is unusual, and is due to the droughty conditions curtailing growth unusually early in the season. Between the yields of grass used for making the hays, the differences just reached significance. Regarding the yield of crude protein, there was a significant difference in favour of the early hay. The data are given in Table XVI.

The yields of starch equivalent were similar from both types of hay, but the yields of digestible true protein and protein equivalent were considerably higher in the early hay, this treatment giving an increase

of 49 and 45 per cent of these constituents respectively, over the ordinary hay.

Table XVI. *Yields of dry matter, crude protein and nutrients in early and ordinary hay. 1934. (Stated as lb. per acre)*

	Early hay	Ordinary hay	Standard error	Significant difference
Dry matter:				
Fresh grass	3256	3668	95.0	373
Hay ex field	3075	3300	103.8	407
Hay ex stack	2932	3285	102.4	402
Crude protein:				
Fresh grass	424	347	20.7	81.2
Hay ex field	396	316	17.9	70.1
Hay ex stack	414	332	11.2	44.0
Digestible true protein	217	146		
Starch equivalent	1487	1564		
Protein equivalent	244	168		
} Hay from stack				

The yield of ordinary hay was not high, amounting to about 34 cwt. per acre. In considering the results, it must be remembered that the season was abnormally dry. Practically no rain fell from mid-May to mid-June, and as the moisture reserve in the soil was low, the growth of the herbage was seriously retarded.

### 1935

Two experiments were conducted in 1935. In the first, the yield of nutrients in early cut hay and the subsequent aftermath was compared with that obtained in hay cut at the normal time. The material used was meadow herbage, as in the previous years.

The second experiment was designed to compare the effect of making "seeds" hay, with a very high percentage of clover, by the ordinary method in use in the south of England, and the pike, coil or tramped-heap method more common in the north. The value of tripods was also tested, but, as will be seen later, under the conditions existing this had to be abandoned.

#### *Experiment 1*

##### *Meadow herbage.*

The experiment was laid out in a field which had been seeded with a permanent mixture in 1932. The herbage contained a high proportion of perennial rye grass and cocksfoot, and a little wild white clover.

It was decided to adhere to the practical methods of haymaking as far as possible. The experimental plots were therefore made larger than in previous years, and a sampling technique was adopted in order to

## 242 *Time of Cutting Hay, and Losses during Haymaking*

estimate the yields of fresh grass at the times of cutting the hays, thus leaving the major part of the swathes undisturbed.

### *Design of experiment.*

Ten plots were measured out, each one-fifth acre in extent, the plots forming a long strip through the field.

The plots, each 44 yards long and 22 yards wide, were divided by a strip 4 yards wide. It was intended to use a single horse-mower with a width of 41 in. to cut the plots, and the width of the plots was so designed that each plot would be twenty swathes wide. The ten plots were divided into five blocks of pairs, and the plots in each block were assigned at random to the two treatments—early hay and ordinary hay.

Some time before the plots were cut, a path 1 yard wide was cut all round the plots, isolating each one. The remaining dividing strip of 2 yards was left to supply fresh grass for the digestibility trials which were carried out on this material.

*Cutting technique.* Each plot was divided on paper in the laboratory into two lengths of 22 yards, and each half divided up into eleven units each of 2 yards. The twenty swathes were then grouped into five strips, each containing four swathes. In each of these strips two samples were taken, each 2 yards long and two swathes wide, one in each half of the plot. The position of these samples was determined at random. Sampling was always started from one end, and the requisite distance was located from this end in one pair of swathes. The 2-yard sample was then isolated by laying two wooden rods across the pair of swathes and measuring the actual width of the two swathes at both ends of the 2-yard length, taking the midpoint between the swathes, and the adjacent swathes as the outside of the cut. These distances were noted, the grass cut along the rods with a sharp knife to give a clean cut, collected and weighed. Each sample was then subsampled for dry-matter determination. In the other pair of swathes, the sample was taken on the second half of the plot at a distance of 22 yards from the first sample. The second subsample for dry matter was bulked with that from the first pair of swathes.

Thus, on each plot, ten samples, each 2 yards long and two swathes wide, were taken at random, and the area sampled was  $1/22$ nd of the total plot area.

The cutting of the early hay was commenced on 27 May, the mower going in one direction only. Immediately a pair of swathes was cut, sampling was carried out, and at no time was the mower allowed to get ahead of the samplers. Cutting had to cease at 11.30 a.m., after two plots

had been cut, owing to heavy rain. The remaining three plots of early hay were cut on 28 May between the hours of 9 a.m. and 12 noon.

The weather immediately after cutting was very bad. The hay was made as well as possible, but for 3 weeks the weather was extremely bad, and the hay had to be cocked and spread out to dry whenever an opportunity was presented. The hay was made separately on each plot, and was put into pikes on 17 June, 20 days after cutting. One pike was built per plot. The pikes were "led" on 3 July, the weight of hay per plot measured, and the necessary samples taken for determination of dry-matter content and digestibility.

There was a small amount of mouldy hay on the outside of the pikes, and this was weighed separately. For the calculations of yield and loss, however, this material was included with the edible hay, since owing to the size of the plots the pikes built were very much smaller than in normal practice, and consequently the proportional waste was greater.

The hay was built into a stack in a hay barn, the material from each plot being separated by strips of hessian or a layer of straw. The stack was opened on 3 December, and the recovered hay weighed and sampled.

The grass for ordinary hay was cut on 24 June, 4 weeks after the early hay cutting. The weather after cutting was fairly good, although during a thunderstorm on the day after the cutting 1.12 in. of rain fell. After this, however, practically no further rain fell while the crop was in field, and the weather was fine and bright.

The hay was put into pikes 4 days after cutting, one on each plot, and the pikes were carted and stacked on 9 July. In one pike only, a little mouldy hay was found.

The stacking procedure was exactly the same as that adopted for the early hay, and the stack was opened on 2 December.

An aftermath cut was taken on 26 June on the plots cut previously for early hay. This was done by cutting two swathes, each exactly 1 yard wide, through the length of the plots with an "Autoscythe", the position of the cuts being fixed at random.

Owing to the lengthy period during which the early hay was lying on the plots, the aftermath growth was checked severely and the herbage was very thin. The determination of the digestibility of this material had to be abandoned owing to the difficulty of collecting it.

### *Composition*

#### *Early hay and aftermath.*

The figures for composition and digestibility are given in Table XVII.

## 244 *Time of Cutting Hay, and Losses during Haymaking*

Table XVII. *Composition, digestibility and digestible nutrients in the fresh grass and early hay, and the composition of the aftermath.* 1935

	Early hay									After-math C.*
	Fresh grass			Ex field			Ex stack			
	C.*	D. (%)	D.N.*	C.*	D. (%)	D.N.*	C.*	D. (%)	D.N.*	
Ether extract	2.32	45.7	1.06	1.87	41.7	0.78	1.86	44.7	0.83	3.14
Fibre	24.07	76.0	18.29	30.23	76.4	23.10	31.75	68.9	21.88	27.09
Crude protein	8.19	56.7	4.64	9.66	45.8	4.42	9.18	41.3	3.79	10.76
Ash	7.09	—	—	7.84	—	—	7.53	—	—	11.31
N-free extractives	58.33	80.3	46.84	50.40	65.9	33.21	49.68	65.6	32.59	47.70
Organic matter	92.91	76.2	70.80	92.16	66.9	61.66	92.47	63.7	58.90	88.69
True protein	6.72	48.8	3.28	8.38	42.7	3.58	8.14	36.3	2.95	9.63
Calcium (CaO)	0.77	—	—	0.79	—	—	0.72	—	—	1.00
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.55	—	—	0.57	—	—	0.58	—	—	0.75
Ratio True protein Crude protein	0.82	—	—	0.87	—	—	0.89	—	—	0.89
Starch equivalent	—	—	62.1	—	—	43.6	—	—	40.4	—
Protein equivalent	—	—	3.96	—	—	4.00	—	—	3.37	—
Dry matter in fresh material	26.0	74.8	—	85.3	64.3	—	84.3	61.3	—	26.2

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

The grass used for making the early hay was not of very good quality, the protein being of the same order as that usually found in average quality ordinary hay.

Marked changes in composition arose during haymaking in the field, and the hay as led from the field shows a large increase in fibre content, an increase in protein content, and a fair decrease in the content of N-free extractives. These changes point to heavy losses of dry matter, and this is confirmed when the yields are considered, as will be seen later. The changes in composition due to curing in the stack were only slight.

All the constituents, with the exception of the fibre, showed a lowering of digestibility as a result of the field drying, the N-free extractives suffering most severely in this regard. The curing in the stack resulted in a decrease in the digestibility of the protein and fibre.

As a result of the changes in composition and digestibility, the starch-equivalent value of the hay is low as compared with that of the original grass, but the protein-equivalent value shows little change.

The aftermath contained high fibre and low protein contents, and was not of very good quality.

### *Ordinary hay.*

The data for composition and digestibility are given in Table XVIII.

The changes in composition are slight, and the figures show that the material was of poor quality, and of low protein content.

The digestibility of the protein was low in the original grass, fell still lower during haymaking in the field, and then rose during curing in the stack to the initial level. The digestibility of the fibre was appreciably higher in the hays than in the grass. The other constituents, apart from the ether extract which fluctuates somewhat, showed only minor changes in digestibility.

Table XVIII. *Composition, digestibility and digestible nutrients in the fresh grass and ordinary hay. 1935*

	Fresh grass			Ordinary hay					
				Ex field			Ex stack		
	C.*	D. %	D.N.*	C.*	D. %	D.N.*	C.*	D. %	D.N.*
Ether extract	2.02	45.0	0.91	1.63	40.6	0.66	1.59	56.9	0.90
Fibre	29.42	59.3	17.45	31.21	68.5	21.38	32.38	67.6	21.89
Crude protein	6.42	36.4	2.34	6.30	28.1	1.77	5.92	37.2	2.20
Ash	8.11	—	—	7.61	—	—	7.28	—	—
N-free extractives	54.03	70.5	38.09	53.25	67.1	35.73	52.83	68.2	36.03
Organic matter	91.89	64.1	58.90	92.39	64.6	59.68	92.72	65.5	60.73
True protein	5.50	31.6	1.74	5.30	24.8	1.31	5.52	31.7	1.75
Calcium (CaO)	0.76	—	—	0.74	—	—	0.58	—	—
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.49	—	—	0.48	—	—	0.46	—	—
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0.86	—	—	0.84	—	—	0.93	—	—
Starch equivalent	—	—	46.9	—	—	41.5	—	—	42.5
Protein equivalent	—	—	2.04	—	—	1.54	—	—	1.98
Dry matter in fresh material	31.3	62.0	—	87.5	61.8	—	85.7	63.3	—

C. = Composition; D. = Digestibility; D.N. = Digestible nutrients.

\* Stated as percentages of the dry matter.

The starch-equivalent value of the grass was low and this value was not greatly depressed in the hays owing to their higher content of digestible fibre.

Little difference is to be seen between the initial and final protein-equivalent values.

#### *Yield data*

The differences between the yields of dry matter from the early and ordinary hay plots were sufficiently great to make a statistical analysis unnecessary. The yields of crude protein, however, were similar and it is seen from Table XIX that, if the aftermath is ignored, the ordinary hay produced more of this constituent than the early hay, the difference just being significant. The inclusion of the aftermath reverses the positions, the early hay treatment giving a significantly higher yield.



## 246 *Time of Cutting Hay, and Losses during Haymaking*

Table XIX. *Yields of crude protein in early and ordinary hay. 1935*  
(*Stated as lb. per acre*)

	Early hay	Ordinary hay	Standard error	Significant difference
Hay	211.3	232.8	5.23	20.5
Hay, including aftermath	263.0	232.8	6.13	24.1

The yields of dry matter, crude protein and nutrients are given in Table XX.

Table XX. *Yields of dry matter, crude protein and nutrients in early and ordinary hay. 1935. (Stated as lb. per acre)*

	Early hay			Ordinary hay	Relative yields, ordinary hay = 100
	1st cut	Aftermath	Total		
Dry matter	2327	471	2798	3991	70
Crude protein	214	51	265	236	112
Digestible true protein	68.6	22.1	90.7	69.8	130
Starch equivalent	940	237	1177	1696	69
Protein equivalent	78.4	25.4	103.8	79.0	131

The statistical examination of the crude protein yields (Table XIX) was made using individual plot yields and crude protein contents, whereas the yields in Table XX were calculated from average values and therefore the small differences noted in the yields of this constituent are to be expected.

The digestibility of the aftermath was not determined as the growth was limited and the collection of the material for feeding the sheep was too laborious. In order to estimate the yields of nutrients in the aftermath, it has been assumed that the digestibility was the same as that of the grass cut for early hay. It is probable that the aftermath is slightly penalized by this assumption.

A good yield of ordinary hay, 42 cwt. per acre, was produced in this year. The early hay yielded only 70 per cent of the dry matter and starch equivalent of the ordinary hay. The early hay yielded 31 per cent more protein equivalent than the ordinary hay, but the yield of this constituent in the latter hay was the lowest recorded in this series of experiments.

### *Experiment 2*

#### *"Seeds" hay.*

In 1934, the field selected for the experiment was in oats, undersown with a 1-year "seeds" mixture of 20 lb. perennial rye grass and 4 lb. broad red clover per acre. The oats had a dressing of 2 cwt. per acre of

Concentrated Complete Fertilizer No. 4, and the maiden seeds received 5 cwt. of basic slag per acre. The field was lightly grazed in the autumn of 1934 and again in the spring of 1935, with ewes and lambs. A dressing of  $1\frac{1}{2}$  cwt. of sulphate of ammonia per acre was applied for the hay, and the crop contained a very high proportion of clover.

The object of the experiment was to compare the yields of nutrients and the losses incurred in three methods of making.

In the first method the hay was made in windrows, and at most built up into small hand-cocks before it was finally led from the field. This, for simplicity, will be denoted as the ordinary process.

The second method was the use of an iron tripod with ventilating ducts after the design of Captain Procter. As will be seen later, the results obtained by this process were unsatisfactory, due largely to overloading of the tripods and to an unfortunate break in the weather immediately after the partly made hay was stacked round the tripods.

The third method, called the "pike" method, entailed the building of tramped heaps, containing 7–10 cwt. of hay, which were built when the hay was three-parts made. These "pikes" were removed from the field in due course on low carts—hay bogies.

### *Design of experiment.*

The experiment was arranged as a random block, with four replications of the three treatments.

The length of the plots was 357 ft., and the width was seven swathes, cut by a 2-horse mower with a knife cut of 4 ft. 6 in. Every effort was made to keep the swathes a standard width. The actual width of each plot was determined by measuring, at each end of the plot, the distance covered by the seven swathes. Three swathes were left between each plot to divide them from each other, and these were removed to facilitate the making of the hay on each plot separately.

For the determination of the initial yield of fresh grass, the produce from the equivalent of one swathe length on each plot was weighed by taking four quarter lengths, distributed at random over the seven swathes. The mowing-machine was not allowed to get far ahead of the samplers, and each quarter-swathe was weighed at once and sampled for determination of dry-matter content.

The cutting of all the plots was carried out on 6 June. The swathes were turned on 11 and 13 June; the tripod hay was built around the metal tripods, one being erected on each plot. On 14 June the material on the other plots was put into windrow. The hay pikes were built on the

## 248 *Time of Cutting Hay, and Losses during Haymaking*

same day, two pikes per plot. The weather during this period was somewhat showery. The ordinary hay was cocked on 15 June, spread out on 17 June, and re-cocked at night. There was heavy rain on 18 June, followed by four sunny days, during which the hay was left in cock. On 24 June the cocks were turned. On the following day the cocks were spread out, but had to be hurriedly re-cocked on account of a thunder-storm with torrential rain. On 26 June the cocks were spread out and re-cocked at night, and were then left in cock the following day.

The carting of the ordinary hay cocks was commenced on 28 June, after the cocks had been spread out a few hours previously. The produce from each plot was weighed and sampled.

The pikes were led on 9 and 10 July, each pike being weighed and sampled. Some waste material was evident in some of the pikes, and this was separated and weighed, but discarded in the calculation of yield data.

The experience with the tripods was unfortunate. Evidently an error was made in attempting to stack too much material round the tripods. As a result, air could not circulate freely through the mass. Immediately after building the hay around the tripods, heavy rain fell and further consolidated the material. The atmosphere remained humid for many days, and little drying occurred inside the heap. The hay in one of the tripods was weighed and sampled on 10 July. The material was wet and very mouldy, and totally unfit for feeding. The other three tripods gave similar results. This result should not be taken as a condemnation of the use of tripods, but shows the difficulties which may arise from too tight packing. The weight of wet hay was so great that the angle iron, of which the tripods were constructed, was buckled. A repetition of the process is essential before the use of tripods can be evaluated.

The hay from all the plots was built into a stack in a Dutch barn, the produce from each plot being kept separate by hessian sheeting.

The stack was opened in December, and the material weighed and sampled.

### *Composition*

The composition of the fresh grass used for haymaking by the ordinary and pike methods, was determined separately on weighted composite samples from the replicated plots. The figures are given in Table XXI, together with the analyses of the hays.

The composition of the two samples of the fresh mixture was almost identical. The changes caused by the drying in the field show the same trend in both treatments. The fibre contents are greatly increased and

all the other constituents, with the exception of the ash and phosphorus, show lowered values. The protein content of the ordinary hay shows a greater drop than that of the piked hay.

Table XXI. *Composition of the crop used for "seeds" hay, and of the hays ex field and ex stack. 1935. (Stated as percentages of the dry matter)*

	Fresh crop		Ordinary hay		Piked hay	
	For ordinary hay	For piked hay	Ex field	Ex stack	Ex field	Ex stack
Ether extract	2.23	2.15	1.02	1.12	1.26	1.24
Crude fibre	26.07	26.14	35.68	37.91	36.25	38.97
Crude protein	14.13	14.14	12.17	11.09	13.03	10.82
Ash	8.42	8.35	8.39	8.12	8.61	8.10
N-free extractives	49.12	49.22	42.74	41.76	40.85	40.87
Organic matter	91.58	91.65	91.61	91.88	91.39	91.90
True protein	10.90	10.99	8.94	9.45	9.82	9.64
Ratio $\frac{\text{True protein}}{\text{Crude protein}}$	0.77	0.78	0.73	0.85	0.75	0.89
Calcium (CaO)	1.82	1.72	1.01	1.09	1.39	1.14
Phosphorus ( $P_2O_5$ )	0.66	0.64	0.67	0.75	0.70	0.82
Dry matter in fresh material	19.7	19.3	83.6	83.6	86.2	84.9

The changes brought about by the curing process in the stack were less severe. The fibre contents of both hays show further increases, and whilst the crude protein contents gave lowered values, the ratio of true protein to crude protein was raised appreciably.

The final figures for the hays showed that the two processes produced material of very similar chemical composition.

#### *Digestibility.*

The digestibility data are given in Table XXII.

'Table XXII. *Digestibility of the crop used for "seeds" hay, and of the hays ex field and ex stack. 1935*

	Fresh crop	Ordinary hay		Piked hay	
		Ex field	Ex stack	Ex field	Ex stack
	%	%	%	%	%
Dry matter	71.2	57.9	56.7	61.3	59.4
Ether extract	76.6	44.7	47.9	37.8	48.5
Crude fibre	67.9	62.3	63.6	68.0	69.0
N-free extractives	78.0	59.6	58.2	61.6	59.4
Organic matter	73.5	59.8	58.7	63.4	61.7
Crude protein	69.0	54.1	46.3	59.3	46.6
True protein	64.1	44.9	36.0	51.7	38.4

The digestibility of the fresh crop was at a satisfactory level, considering the rather mature state of the material.

## 250 *Time of Cutting Hay, and Losses during Haymaking*

Drying in the field resulted in a loss in digestibility of all the constituents, the most serious losses falling on the true and crude protein and the N-free extractives. Hay made by both processes showed these losses, but in piked material they were less severe.

The only constituents to show any marked change in digestibility during the curing process in the stack, were the true and crude protein. The values for these constituents were lowered, the piked hay suffering more severely in this regard.

On the whole, the material made by the pike method was of slightly higher digestibility than that made by the ordinary method, when the final products are considered.

### *Digestible nutrients.*

The calculated digestible nutrients contained in the various crops are given in Table XXIII.

Table XXIII. *Digestible nutrient content of the crop used for "seeds" hay, and of the hays ex field and ex stack. 1935. (Stated as percentages of the dry matter)*

	Fresh crop (Average of two treatments)	Ordinary hay		Piked hay	
		Ex field	Ex stack	Ex field	Ex stack
Ether extract	1.5	0.5	0.5	0.5	0.6
Fibre	17.7	22.2	24.1	24.7	26.9
Crude protein	9.8	6.6	5.1	7.7	5.0
N-free extractives	38.4	25.5	24.3	25.2	24.3
Organic matter	67.3	54.8	53.9	57.9	56.7
True protein	7.0	4.0	3.4	5.1	3.7
Dry matter	71.2	57.9	56.7	61.3	59.4
Starch equivalent	57.3	31.7	30.7	34.5	33.2
Protein equivalent	8.4	5.3	4.3	6.4	4.4

The hays contained more digestible fibre than the original grass, but considerably less of all the other digestible nutrients. The starch-equivalent values of the hays were lowered, this being due to the decreased digestibility of the constituents and to the use of a higher fibre correction.

The two processes of haymaking have given products of very similar nutritive value.

### *Yield data*

A statistical examination was made of the yields of dry matter and crude protein at the various stages of the experiment, and the figures are given in Table XXIV.

Table XXIV. *Yields of dry matter and crude protein from the fresh crop and the hays. 1935. (Stated as lb. per acre)*

	Ordinary hay	Piked hay	Tripod hay	Standard error	Significant difference
Dry matter:					
Fresh crop	5982	6265	6201	138.9	481
Hay ex field	4309	4983	—	103.7	467
Hay ex stack	3973	4484	—	124.2	559
Crude protein:					
Fresh crop	849	887	883	20.2	70
Hay ex field	509	640	—	15.0	67
Hay ex stack	433	464	—	11.4	51

The area used in the experiment was uniform, there being no significant difference between the treatments in the yields of dry matter and crude protein in the fresh crop. The pike method of field drying resulted in a significantly higher yield of dry matter and crude protein, as compared with the ordinary method. In the stack, however, the piked material suffered greater changes, and as a result the final yields show no significant difference between the two treatments.

The yields of nutrients given in Table XXV have not been examined statistically, as the data are insufficient, but it is evident that the yields from the piked material are appreciably greater than those from the ordinary material.

Table XXV. *Yields of nutrients from ordinary and piked hays. 1935. (Stated as lb. per acre)*

	Ordinary hay	Piked hay
Starch equivalent	1220	1489
Protein equivalent	170	196
Digestible true protein	135	166

### *Summary of yield data 1931-5*

The yield data for the 5 years during which the experiment was in progress are summarized in Table XXVI.

It would appear that the weather conditions at the usual haymaking time are generally more favourable than those obtaining a few weeks earlier, when the early hay should be cut.

The average production of the ordinary hay was 3427 lb. per acre of dry matter or 36 cwt. of fresh hay, and this exceeds that of the early hay by 992 lb. per acre, or 9½ cwt. of fresh hay. The production of starch equivalent is greater in the ordinary hay by 249 lb. per acre, but the early hay yielded 47 lb. per acre more protein equivalent.

## 252 *Time of Cutting Hay, and Losses during Haymaking*

Table XXVI. *Summary of hay yields, including the aftermath cut in the early hay treatment. 1931-5. (Stated as lb. per acre)*

Year	Type	Weather conditions	Dry matter	Crude protein	Starch equivalent	Protein equivalent	Digestible true protein
1931	Early hay	Poor	2064	229	919	104	88
	Ordinary hay	Fairly good	3237	234	1207	101	92
1932	Early hay	Excellent	1690	215	876	111	101
	Ordinary hay	Poor	2217	184	905	84	75
1933	Early hay	Fair	2693	385	1427	245	229
	Ordinary hay	Excellent	4403	347	1757	141	134
1934	Early hay	Very good	2932	414	1487	244	217
	Ordinary hay	Excellent	3285	332	1564	168	146
1935	Early hay	Very poor	2798	265	1177	104	91
	Ordinary hay	Good	3991	236	1696	79	70
Av.:	Early hay	—	2435	302	1177	162	145
	Ordinary hay	—	3427	267	1426	115	103
“Seeds” hay:							
1935	Ordinary hay	Poor	3973	433	1220	170	135
	Piked hay	Poor	4484	464	1489	196	166

In considering the total productivity per acre of grassland under the two treatments, the main comparison to be made is between the relative values of 249 lb. of starch equivalent and 47 lb. of protein equivalent. A number of other factors must be taken into account if the economics of the two treatments are considered, e.g. the increased handling costs of the larger bulk of the ordinary hay and of the manure obtained therefrom.

The preceding summary has dealt with the total productivity of the areas under treatment. The early hay treatment therefore includes an aftermath, which under practical conditions would be grazed by cattle, and its value to the farmer will vary according to the condition of his pastures. In general this aftermath is available at a time when there is a fairly plentiful supply of herbage. Since the purpose of haymaking is to conserve fodder for use during the winter months, it will be of value to examine the yields of hay from the treatments, excluding the aftermath cut for the early hay treatment. From the ordinary hay the dry-matter, starch-equivalent and protein-equivalent yields were 3427, 1426 and 115 lb. per acre respectively, whilst the early hay gave 1958, 904 and 126 lb. respectively. The ordinary hay therefore conserved 522 lb. more starch equivalent and only 11 lb. less protein equivalent than the early hay.

It would appear that the method of cutting the hay at an early stage of growth (some 4-5 weeks earlier than normal) cannot compete with the ordinary method when the yields of nutrients in the made hays alone are considered.

*Losses arising during haymaking**Meadow hay.*

The losses which occurred during the field-drying process and the curing process in the stack are given in a summarized form in Table XXVII. The full data concerning the statistical findings on the losses of dry matter and crude protein are given in the Appendix.

The influence of the weather conditions on the losses occurring during the drying process in the field is clearly seen from the table. Under

Table XXVII. *Summary of haymaking losses. 1932-5.*  
(*Stated as percentages of the original material*)

Gains stated with positive sign							
Year	Weather conditions	Type		Dry matter	Crude protein	Starch equivalent	Protein equivalent
1932	Excellent	Early hay	In field	20.5	17.6	31.4	24.7
			In stack	5.0*	0.4	13.7	19.4
			Total	25.5	18.0	45.1	44.1
	Poor	Ordinary hay	In field	33.9	51.3	46.7	75.4
			In stack	2.8	+ 9.8	1.7	+ 21.7
			Total	36.7	41.5	48.4	53.7
	Fair	Early hay	In field	15.5	12.7	36.1	30.0
			In stack	5.5	3.8	2.4	+ 0.8
			Total	21.0	16.5	38.5	29.2
1933	Excellent	Ordinary hay	In field	12.2	11.5	25.6	24.0
			In stack	5.2	3.1	7.4	4.7
			Total	17.4	14.6	33.0	28.7
	Very good	Early hay	In field	5.6	6.8	19.4	7.7
			In stack	4.4	+ 4.4	3.6	1.2
			Total	10.0	2.4	23.0	8.9
	Excellent	Ordinary hay	In field	10.0	9.0	21.7	10.0
			In stack	0.4	+ 4.5	1.3	7.0
			Total	10.4	4.5	23.0	17.0
1935	Very poor	Early hay	In field	30.5	20.2	51.2	29.8
			In stack	6.0	9.0	7.5	16.1
			Total	36.5	29.2	58.7	45.9
	Good	Ordinary hay	In field	1.2	2.4	12.6	25.4
			In stack	14.4	22.0	10.9	+ 7.3
			Total	15.6	24.4	23.5	18.1
	Average:	Early hay	In field	18.0	14.3	34.5	23.0
			In stack	5.2	2.2	6.8	9.0
			Total	23.2	16.5	41.3	32.0
"Seeds" hay:		Ordinary hay	In field	14.3	18.5	26.7	33.7
			In stack	5.7	2.7	5.3	+ 4.3
			Total	20.0	21.2	32.0	29.4
		Piked hay	In field	28.0	40.0	60.1	54.5
			In stack	5.6	9.0	4.3	11.5
			Total	33.6	49.0	64.4	66.0
	1935 Poor		In field	20.5	27.8	52.1	39.4
			In stack	8.0	19.8	6.4	23.1
			Total	28.5	47.6	58.5	62.5

\* An assumed value.



## 254 *Time of Cutting Hay, and Losses during Haymaking*

favourable conditions the losses of dry matter and starch equivalent in the ordinary hay were approximately 8 and 20 per cent respectively, and under poor conditions the values were 34 and 47 per cent respectively. Similarly with the early hay, the losses under favourable conditions were 13 and 25 per cent, and under poor conditions 23 and 44 per cent for the dry matter and starch equivalent respectively.

The losses arising during the curing process in the stack are generally of minor importance, although in certain circumstances these may vary to a major degree. For example, in 1932 the early hay showed losses in the stack of 14 and 19 per cent of starch equivalent and protein equivalent respectively. These figures are much above the average, and are probably due to the hay being stacked somewhat too fresh, the digestibility of the material being lowered by the resulting fermentation. Also, in 1932 the ordinary hay showed an increase in protein equivalent in the stack owing to the raising of the digestibility of the protein fraction of the material.

Comparing the average losses in the two types of hay, it is seen that the early hay suffered slightly greater losses of dry matter and protein equivalent, and an appreciably greater loss of starch equivalent, than the ordinary hay. The poorer weather conditions at the time of making the early hay probably account for these differences.

### *“Seeds” hay.*

The losses in the “seeds” hay were very high. The weather conditions were poor, and it is evident from the figures that the piking treatment, as compared with the ordinary treatment, has to some extent saved the material from the effects of the weather. In the stack, however, the piked hay showed greater losses, and as a result there was little difference between the total losses by the two treatments.

The results for losses of nutrients in the series of experiments discussed above show good agreement with the figures already quoted from the Swiss work of Wiegner<sup>(1)</sup>. It should also be remembered that the period covered by these experiments included 2 years (1933 and 1934) in which the conditions for haymaking were well above the average. Despite this both types of hay lost from one-quarter to one-third of the starch equivalent under excellent conditions for curing in the field. Over a normal range of years the average losses would probably exceed the average of this series.

## SUMMARY

Experiments have been carried out from 1930 to 1935, to investigate the composition, digestibility and yield of meadow hay cut at a normal time, and also of hay cut some 3-5 weeks earlier. Due consideration has been given to the "aftermath" growth occurring on the areas cut for the early hay between the times of cutting of the early and ordinary hay.

As was expected, the early hay, being less mature, was of better composition and digestibility than the ordinary hay. The average crude protein contents were 12.2 and 7.9 per cent of the dry matter respectively.

The yield data, average of 5 years, are summarized below, stated as lb. per acre, the early hay values including the aftermath yields.

	Early hay + aftermath	Ordinary hay
Dry matter	2435	3427
Crude protein	302	267
Digestible true protein	145	103
Starch equivalent	1177	1426
Protein equivalent	162	115

The ordinary hay yielded 249 lb. per acre more starch equivalent and 47 lb. less protein equivalent than the early hay.

Considering only the material conserved for winter fodder, that is excluding the aftermath on the early hay areas, the early hay yields of dry matter, starch equivalent and protein equivalent were 1958, 904 and 126 lb. per acre respectively, and the ordinary hay therefore conserved 522 lb. more starch equivalent and only 11 lb. less protein equivalent.

The losses, average of 4 years, occurring during haymaking and curing in the stack are summarized below, stated as percentages of the original fresh material.

Type		Dry matter	Starch equivalent	Protein equivalent
Early hay	In field	18.0	34.5	23.0
	In stack	5.2	6.8	9.0
	Total	23.2	41.3	32.0
Ordinary hay	In field	14.3	26.7	33.7
	In stack	5.7	5.3	+ 4.3*
	Total	20.0	32.0	29.4

\* Gain.

Adverse weather conditions caused high losses, the highest losses of dry matter, starch equivalent and protein equivalent being 36.7, 58.7 and 53.7 per cent respectively. The weather conditions were, in general, more

## 256 *Time of Cutting Hay, and Losses during Haymaking*

favourable at the normal haymaking periods than at the periods selected for making the early hay.

In 1935 a comparison was made of two methods of making "seeds" hay. One method was ordinary windrow drying, and the other the tramped heap or pike.

The composition of the two hays was very similar. The digestibility of the piked hay was very slightly higher than that of the ordinary hay.

No significant difference was found between the two hays in the yields of dry matter and crude protein, but the piked hay gave a greater yield of starch equivalent and protein equivalent.

Poor weather conditions caused heavy losses in the field. These were somewhat greater in the hay made by the ordinary method, but in the stack the piked hay gave slightly higher losses, particularly as regards protein equivalent.

The total losses of starch equivalent and protein equivalent were approximately 60 per cent.

### ACKNOWLEDGEMENTS

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## APPENDIX

*Standard errors on losses of dry matter and crude protein*

1932

		Dry matter	
Type		Loss %	Standard error %
Early hay, in field		20.5	9.0
Ordinary hay, in field		33.9	1.9
Ordinary hay, in stack		2.8	—

1933

		Mean lb. per acre	Standard error of mean lb.	Mean %	Standard error of mean %
		Dry matter			
Early hay	In field	413	36	15.5	1.3
	In stack	148	19	5.5	0.7
	Total	561	27	21.0	1.0
Ordinary hay	In field	649	161	12.2	3.0
	In stack	276	41	5.2	0.8
	Total	925	158	17.4	3.0
		Crude protein			
Early hay	In field	45.8	7.8	12.7	2.2
	In stack	13.6	4.6	3.8	1.3
	Total	59.4	5.3	16.5	1.5
Ordinary hay	In field	47.1	15.0	11.5	3.7
	In stack	12.5	8.7	3.1	2.1
	Total	59.6	14.8	14.6	3.6

1934

		Dry matter			
Early hay	In field	181	72.3	5.6	2.2
	In stack	143	47.6	4.4	1.5
	Total	324	72.8	10.0	2.2
Ordinary hay	In field	368	125.1	10.0	3.4
	In stack	15	22.5	0.4	0.6
	Total	383	134.9	10.4	3.7
		Crude protein			
Early hay	In field	28.9	10.2	6.8	2.4
	In stack	-18.8	9.7	-4.4	2.3
	Total	10.1	11.5	2.4	2.7
Ordinary hay	In field	31.3	27.7	9.0	8.0
	In stack	-15.8	5.9	-4.5	1.7
	Total	15.5	24.4	4.5	7.0

# 258 *Time of Cutting Hay, and Losses during Haymaking*

## APPENDIX (*continued*)

### 1935. *Meadow hay*

Type		Mean lb. per acre	Standard error of mean lb.	Mean %	Standard error of mean %
Dry matter					
Early hay	In field	1117	179	30.5	4.9
	In stack	218	59	6.0	1.6
	Total	1335	136	36.5	3.7
Ordinary hay	In field	57	87	1.2	1.8
	In stack	682	87	14.4	1.8
	Total	739	105	15.6	2.2
Crude protein					
Early hay	In field	60.2	8.0	20.2	2.7
	In stack	26.7	5.7	9.0	1.9
	Total	86.9	13.1	29.2	4.4
Ordinary hay	In field	7.3	8.7	2.4	2.8
	In stack	67.9	6.2	22.0	2.0
	Total	75.2	11.4	24.4	3.7

### 1935. "*Seeds*" hay

Dry matter					
Ordinary hay	In field	1673	133	28.0	2.2
	In stack	336	140	5.6	2.3
	Total	2009	161	33.6	2.7
Piked hay	In field	1282	93	20.5	1.5
	In stack	499	100	8.0	1.6
	Total	1782	110	28.5	1.8
Crude protein					
Ordinary hay	In field	340	21.6	40.0	2.5
	In stack	76	12.4	9.0	1.5
	Total	416	17.5	49.0	2.1
Piked hay	In field	246	6.3	27.8	0.7
	In stack	176	27.4	19.8	3.1
	Total	422	31.1	47.6	3.5

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# THE SOLUBILITY OF SOIL PHOSPHORUS AND OTHER PHOSPHORUS COMPOUNDS IN SODIUM HYDROXIDE SOLUTIONS

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THE problem of the fractionation of soil phosphorus has been the subject of many investigations. The main approach has been through the study of its solubility in acid solutions varying in reaction from neutrality to that of concentrated acids. Its solubility in weakly buffered solutions is the basis of most of the methods in current use for the estimation of available phosphorus. Attempts have also been made to differentiate the various soil phosphorus compounds by the use of selected buffered acidic solutions having no appreciable action on some materials whilst dissolving considerable amounts of others<sup>(1)</sup>. Very little attention, on the other hand, has been given to the solubility of soil phosphorus in solutions on the alkaline side of neutrality, though it is well known that such extracts contain appreciable amounts of phosphorus. Das<sup>(2)</sup> proposed a method for determining available  $P_2O_5$  by extracting with a weak solution of sodium carbonate, but this method does not appear to have been widely adopted. Treatment of soils with strong alkaline solutions results in a partial decomposition of the colloidal complex, large amounts of organic and mineral matter being brought into solution. These extracts may contain a considerable proportion of the soil phosphorus. When mineral phosphates such as apatite, Nauru and Gafsa phosphates, or basic slag are treated in a similar manner the amount of phosphorus extracted is very small—almost negligible (see Table III). It was, therefore, considered that an investigation into the amounts of phosphorus extracted from soils and phosphatic minerals by alkaline solutions might provide some information regarding the nature of soil phosphorus compounds.

## EXPERIMENTAL

Preliminary experiments with sodium hydroxide had shown that appreciable amounts of phosphorus could be extracted from soils with relatively dilute solutions. In order to investigate this in greater detail

it was first necessary to decide on methods of extraction and estimation which, when used, would give reproducible and trustworthy results. This work consisted of two stages: (1) the extraction of the soil with sodium hydroxide solution, and (2) the estimation of  $P_2O_5$  in the alkaline extract.

*Methods of extraction and estimation*

(a) *Extraction with sodium hydroxide.* As the object was to extract the maximum amount of  $P_2O_5$ , the soil and sodium hydroxide solution were boiled together in a beaker. In general, it was found that the boiling could not be continued beyond about  $2\frac{1}{2}$ –3 hours because of the development of a tendency to violent bumping. Also, in a few cases where comparisons were made, no significant difference was shown between the amounts extracted after 1 and 3 hours' boiling. The method finally used to obtain the results recorded in Table II was as follows.

10 g. of soil and 200 c.c. of sodium hydroxide solution were heated to boiling in a 400 c.c. pyrex beaker. The beaker was covered with a clock glass and placed on a hot plate adjusted so as to keep the mixture in gentle ebullition. It was kept there for  $2\frac{1}{2}$ –3 hours with occasional stirring and addition of water to maintain the volume at a fairly constant level. As quite large changes in the concentration of the NaOH (see Table II) were needed to cause a significant difference in the amount of  $P_2O_5$  extracted, it was not necessary to keep the volume absolutely constant. Near the end of the digestion the volume was allowed to diminish slightly so as to allow for the use of a small quantity of water to wash the mixture afterwards into a graduated cylinder. When the digestion was complete, the contents of the beaker were allowed to cool and transferred into a graduated cylinder with enough water to make the volume of liquid up to 200 c.c. The contents of the cylinder were then thoroughly shaken and allowed to stand overnight. On the following day, in most cases, an aliquot portion of the supernatant liquid could be pipetted or syphoned off and used immediately for the determination of  $P_2O_5$ . In others it was necessary to remove suspended matter by filtration before proceeding with the determination.

(b) *Determination of  $P_2O_5$  in the alkaline extract.* The soil extract with sodium hydroxide contains large amounts of organic matter and silica which must be removed before the  $P_2O_5$  can be determined. It was essential that a fairly rapid technique should be developed for this purpose if accurate and trustworthy data were to be obtained for a number of soils. At first the extracts were boiled with aqua regia, evaporated to dryness, etc., in the usual manner to dehydrate the silica. The residue was then

taken up in dilute sulphuric acid and the phosphate in the extract determined by precipitation with ammonium molybdate. It was found that, although concordant results were obtained with duplicates, some phosphorus still remained in the residue after extracting with dilute sulphuric acid, presumably in the small amounts of organic matter which resisted oxidation by aqua regia. It was found possible to eliminate this error by treatment with concentrated nitric acid or aqua regia followed by evaporation to dryness and strong ignition prior to the separation of the silica. As this procedure was slow and great care had to be exercised to prevent loss by spitting during the various operations it was decided to try alternative, and more rapid, methods for removing the organic matter and silica. Digestion with a mixture of sulphuric and nitric acids with the addition of a few grams of potassium sulphate and a little copper sulphate was found to be the most efficient mixture for oxidizing the organic matter. Violent bumping with the consequent risk of losing some of the solution was liable to occur, in many cases, when the potassium sulphate was omitted. The operation was carried out in an ordinary 500 c.c. Kjehdahl digestion flask and the oxidation completed in 30-45 min. At the same time, the silicic acid was dehydrated by the concentrated sulphuric acid and could, after dilution with water, be separated from the solution by filtration.

Determination of  $P_2O_5$  in mixtures of sodium silicate and ammonium phosphate which had been subjected to this treatment showed that trustworthy results were obtained.

The details of the procedure with extracts obtained as described under (a) are as follows: 25, 50 or 100 c.c. (depending on the amount of  $P_2O_5$  present) of the sodium hydroxide extract were measured into a 500 c.c. Kjehdahl flask. Enough concentrated sulphuric acid to neutralize the sodium hydroxide and to give 10-12 c.c. excess acid was added, followed by 10 c.c. concentrated nitric acid, about 5 g. of potassium sulphate, and a small amount of copper sulphate. The flask was heated until the organic matter had been oxidized and the sulphuric acid reached the fuming stage. The heating was continued for a further 10-15 min. to ensure the dehydration of the silica. The contents of the flask were allowed to cool, about 50 c.c. of cold water added, the whole washed on to a filter, and the filtrate collected in a 400 c.c. beaker. The residue on the filter was washed several times with water. The filtrate and washings were neutralized with ammonia, 20 c.c. of concentrated nitric acid and 30 c.c. of 50 per cent ammonium nitrate solution were added, the solution was heated to about 70° C., and 60 c.c. of 3 per cent ammonium molybdate



solution were stirred in. The beaker containing the solution and yellow precipitate was placed on the top of a steam oven, covered with a clock glass, and allowed to stand overnight at about 45° C. The next morning it was taken off, allowed to cool, and its contents filtered through a weighed Gooch crucible, the precipitate being transferred into the crucible and washed with 1 per cent nitric acid solution. After being dried in the oven it was carefully ignited to the blue-black stage and weighed.

The factor for converting the weight of the blue-black precipitate into its equivalent of  $P_2O_5$  appears to vary slightly with the conditions of precipitation. Under conditions identical with those described above it was found that the factor required for pure phosphate solutions was 0.038. This was used for calculating the data reported here.

Using the above methods, the following points were investigated:

(1) The influence of sodium hydroxide solution concentration on the amounts of  $P_2O_5$  extracted from (a) acid soils (Table II A), and (b) soils containing free calcium carbonate (Table II B).

(2) The effect of removing exchangeable calcium from the soil colloidal complex on the amount of  $P_2O_5$  extracted by sodium hydroxide solutions. This was carried out by leaching with ammonium acetate before adding the sodium hydroxide (Tables II A and II B).

(3) The amounts of  $P_2O_5$  extracted by sodium hydroxide solutions from phosphorus minerals and compounds (Table III).

Table I. *Particulars of soils used*

Soil no.	Organic carbon %	pH	Exchangeable CaO, m.e. per 100 g.	Description
<b>Acid soils</b>				
I	3.00	4.7	0.43	Podsol B-horizon. Caernarvonshire
II	3.94	5.3	6.09	Light loam from granitic drift. Aberdeenshire
III	3.06	5.7	8.16	Shaly loam. Caernarvonshire
IV	5.40	5.0	5.00	Humus light loam. Caernarvonshire
V	0.49	5.5	0.31	Loam from acid igneous material. India
VI	22.20	4.2	1.02	Light peaty soil. Glamorganshire
VII	1.93	5.6	8.50	Carrington silt loam. Iowa, U.S.A.
<b>Carbonate soils</b>				
			CaCO <sub>3</sub> % per 100 g.	
VIII	4.48	7.4	0.2	Garden soil. Glamorganshire
IX	5.80	7.3	1.45	Garden soil from shaly material. Caernarvonshire
X	2.33	7.95	2.85	Light shaly loam. Denbighshire
XI	0.28	7.8	1.8	Green-sand subsoil. Berkshire
XII	2.33	7.8	19.2	Chalk soil. Berkshire

*Soils used in the investigation*

Table I gives a description of the soils which were examined by the above method and for which results are given in Tables II A and II B.

Table II A. *Acid soils. Extraction of soil phosphorus with solutions of sodium hydroxide of varying strength*

Soil no.	% total $P_2O_5$ by $H_2SO_4$ method	% $P_2O_5$ extracted by NaOH solutions					% $P_2O_5$ extracted by $NH_4Ac$	Difference between total $P_2O_5$ and amount extracted by 5 % NaOH from the ammonium soil + that extracted by $NH_4Ac$
		1 %	1 %	2 %	5 %	10 %		
I	0.331	0.196	0.227	0.212	0.253	0.256	0.269	—
I*	0.331	0.221	0.229	0.245	0.257	0.266	—	0.002
II	0.491	0.420	0.432	0.438	0.444	0.450	0.450	—
II*	0.491	0.450	0.432	0.435	0.444	0.456	—	0.0015
III	0.366	0.232	0.259	0.280	0.309	0.316	0.317	—
III*	0.366	0.320	—	0.328	0.331	0.337	—	0.0045
IV	0.513	0.432	0.452	0.481	0.487	0.498	0.490	—
IV*	0.513	0.448	0.481	0.487	0.496	0.498	0.496	0.002
V	0.126	0.1065	0.1155	0.119	0.119	0.1195	0.1065	—
V*	0.126	0.118	—	0.1215	0.1215	0.1215	—	0.001
VI	0.113	0.085	0.099	0.103	0.104	0.105	0.099	—
VI*	0.113	0.102	0.096	0.096	0.099	0.102	—	0.003
VII	0.104	—	—	0.084	0.086	0.090	—	—
VII*	0.104	0.095	0.090	0.090	0.088	0.090	—	0.0015

\* Ammonium soils (10 g. leached with 1 litre of N ammonium acetate).

Table II B. *Carbonate soils. Extraction of soil phosphorus with solutions of sodium hydroxide of varying strength*

Soil	% total $P_2O_5$ by $H_2SO_4$ method	% $P_2O_5$ extracted by NaOH solution:				Difference between total $P_2O_5$ and the amount extracted by 5 % NaOH from the ammonium soils
		2 %	5 %	10 %	20 %	
VIII	0.260	0.169	0.206	0.209	0.228	—
VIII*	0.260	0.222	0.210	0.216	0.225	0.050
IX	0.697	0.103	0.148	0.223	—	—
IX*	0.697	0.203	0.238	0.268	—	0.459
X	0.142	0.079	0.108	0.113	—	—
X*	0.142	0.091	0.117	0.128	—	0.025
XI	1.760	0.015	0.021	0.022	0.027	—
XI*	1.760	0.032	0.041	0.039	0.038	1.720
XII	0.296	0.070	0.070	0.079	0.111	—

\* Ammonium soil (10 g. leached with 1 litre of N ammonium acetate. The  $P_2O_5$  dissolved by the ammonium acetate was not determined for all the soils. As it is not more than 0.01 per cent it was neglected in calculating the figures in the last column).

The data in Table IIA show that even the weakest concentration of sodium hydroxide extracts the greater part of the phosphorus from these acid soils, and that with strengths from 2 per cent upwards the amount extracted is constant or shows a slight tendency to increase. This suggests the presence of two types of phosphorus compounds: (a) com-

pounds readily soluble in soda, (b) compounds with a very low soda solubility. At the lower concentrations of sodium hydroxide it is clear that the presence of exchangeable calcium affects the extraction of the phosphorus. The figures for the ammonium soils show a closer constancy for all concentrations and at the higher concentrations are—within the limits of experimental error—identical with those for the untreated soils. Thus, when the exchangeable calcium has been removed, an almost constant amount of phosphorus is extracted from acid soils by sodium hydroxide solutions of strengths from 2 per cent upwards. A comparison of the amount extracted by sodium hydroxide with the total phosphorus determined by the sulphuric acid method of McLean<sup>(9)</sup> shows that in most of these soils over 90 per cent of their phosphorus is soda-soluble. The figures in Table IIA suggest that the phosphorus dissolved by 5 per cent sodium hydroxide, from the ammonium soils, gives a fair measure of the amount of the total soda-soluble phosphorus. The amount of soda-insoluble phosphorus can now be approximately ascertained by subtracting the soda-soluble phosphorus, plus the phosphorus dissolved in the ammonium acetate solution, from the total. The figures obtained in this way are given in the last column of Table IIA.

The interpretation of the results for soils containing free calcium carbonate (Table II B) presents considerable difficulties. The soils show a lack of uniformity in their behaviour with sodium hydroxide solutions. The figures for two of them, VIII and X, are similar to those for the acid soils, viz. a high proportion of the total phosphorus is soda-soluble and a fairly constant amount is extracted by different strengths of sodium hydroxide solutions from the ammonium soils. The other soils indicate only a small proportion of soda-soluble phosphorus even after conversion to the ammonium soil. The leaching with ammonium acetate results in the removal of the calcium from the exchange complex but removes only a part of the calcium carbonate. But for this latter fact, it would be justifiable to conclude that these soils contain a high percentage of soda-insoluble phosphorus. It is highly probable that they do contain considerable amounts of calcium phosphates of the apatite type, but this is not proved by the available experimental data, as boiling certain soda-soluble phosphate compounds (see below) with sodium hydroxide solution in the presence of calcium carbonate results in the precipitation of the soluble phosphorus as insoluble calcium phosphates. For example, 80–90 per cent of the phosphorus of aluminium phosphate (shown in Table III to be completely soda-soluble) is rendered insoluble in sodium hydroxide solution when boiled in the presence of calcium carbonate.

Ferric phosphate and laboratory samples of calcium phosphates behave in a similar manner. It is, therefore, not possible to distinguish between the soda-soluble and soda-insoluble phosphorus compounds when the soils contain free calcium carbonate.

The high percentage of soluble phosphorus in soils VIII and X may be due partly to a smaller amount of carbonate in a finely divided condition, and partly to much of the phosphorus being combined with the organic matter and not precipitated from sodium hydroxide solution when boiled with calcium carbonate. Some support for the latter view was obtained by extracting two of the acid soils with 5 per cent sodium hydroxide solution in the presence of 5.0 per cent  $\text{CaCO}_3$ . The amount of phosphorus extracted from soil II, which gives a very dark coloured extract with sodium hydroxide, was about 90 per cent of the total soluble phosphorus, whilst from soil V, containing very little extractable organic matter, only about 30 per cent of its soluble phosphorus was obtained. The evidence, however, is too scanty and inconclusive to warrant anything further on these lines than the suggestion that phosphorus combined with organic matter is not easily precipitated by calcium carbonate from sodium hydroxide solution. Further experimental work on this point is in progress.

With regard to the general question of the solubility of soil phosphorus compounds in sodium hydroxide, the results indicate that in acid soils it is possible by the sodium hydroxide method to distinguish two types of compounds, the soda-soluble and the soda-insoluble; but for the reasons stated above this cannot at present be done when soils contain free calcium carbonate.

#### *Experiments with phosphorus minerals and compounds*

In order to obtain some knowledge of the type of phosphorus compounds insoluble in sodium hydroxide solution a number of phosphorus minerals, compounds and fertilizers were extracted with 5 per cent sodium hydroxide solution by the method used for soils. The results are shown in Table III.

The results may be divided into two groups: (1) low solubility and (2) high solubility. The calcium mineral phosphates, basic slag and the ordinary bone manures have a very low solubility, whilst ferric and aluminium phosphates, the iron and aluminium phosphate minerals, vivianite, dufrenite and wavellite, bone ash, laboratory calcium phosphate and "fertiphos" have a high solubility. The actual weight of  $\text{P}_2\text{O}_5$  extracted from 1 g. of the calcium mineral phosphates, basic slag, and

Table III. *Solubility of phosphorus compounds and minerals in sodium hydroxide*

Material	Weight taken in g.	% P <sub>2</sub> O <sub>5</sub>	
		Total	Extracted by 5 % NaOH
Apatite	1.0	42.7	0.091
Apatite (leached)*	1.0	42.7	0.076 (0.285)*
Nauru phosphate	1.0	38.95	Trace
Nauru phosphate (leached)*	1.0	38.95	0.198 (0.395)*
Gafsa phosphate	1.0	27.75	Trace
Gafsa phosphate (leached)*	1.0	27.75	0.061 (0.27)*
Curacao phosphate	1.0	40.2	0.166
Vivianite	0.5	3.65	3.54
Wavellite	0.1	19.4	18.4
Dufrenite	0.5	2.97	1.28
Basic slag	1.0	15.02	0.030
Basic slag (leached)*	1.0	15.02	0.129 (4.27)*
Bone meal	1.0	22.25	0.116
Steam bone flour	1.0	30.1	0.638
Bone ash	1.0	38.8	11.4
Phosphate guano	1.0	19.5	3.95
"Fertiphos"	1.0	23.3	15.6
Lab. Ca phosphate S1	1.0	44.1	6.82
Lab. Ca phosphate S2	0.1	53.8	17.2
Lab. Fe phosphate	0.1	39.1	38.0
Lab. Al phosphate	1.0	34.7	33.0

\* Leached with 500 c.c. ammonium acetate previous to extraction with sodium hydroxide; the amount of P<sub>2</sub>O<sub>5</sub> dissolved by the ammonium acetate given in brackets.

bone meal was so small that they may be regarded as being practically insoluble in sodium hydroxide. Ferric and aluminium phosphates appear to be completely soluble. The samples of vivianite, wavellite and dufrenite available for this work were very impure and contained other minerals, but there seems little doubt from the figures that the iron or aluminium phosphates in them are soluble in 5 per cent sodium hydroxide solutions. During recent years, as the result of examination by X-ray and other methods, it has been shown that natural calcium phosphates possess the general apatite structure—Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub> X<sub>2</sub>, where X<sub>2</sub> may be (F<sub>2</sub>), (Cl<sub>2</sub>), (OH)<sub>2</sub>, CO<sub>3</sub> or (O) or mixtures of these, and that the calcium phosphate which is stable in contact with water at the reaction of soils is the hydroxyapatite. The composition of basic slag appears to be more complex, containing various forms of calcium phosphates and silicophosphates. Crowther<sup>(1)</sup> has summarized the position in the light of recent investigations, and states that in low-soluble open hearth slag the greater part of the phosphorus is present as fluorapatite and that it is possible that some modern slags owe their insolubility to hydroxyapatite, as it has been shown that calcium tetraphosphate kept at about 1100° C.

is converted to an apatite by absorption of moisture from the atmosphere.

It would, therefore, seem that the phosphorus compounds with a low sodium hydroxide solubility are those with an apatite structure. The figures from the two samples of laboratory calcium phosphates show that a fraction of each is soluble in sodium hydroxide. They are obviously impure complexes containing a higher percentage of phosphorus than tricalcium phosphate or hydroxyapatite, and probably contain some dicalcium phosphate. This would account for the considerable solubility in sodium hydroxide.

#### GENERAL DISCUSSION

It has been shown in the foregoing experiments that of the minerals examined only those known to possess an apatite structure, including basic slag (whose phosphorus compounds are closely related to apatite), show a low solubility in sodium hydroxide. Although calcium, iron, and aluminium phosphates are the usual parent materials of soil phosphorus, it would not be correct to assume that the difference between the total phosphorus and the soda-soluble phosphorus gives an exact measure of the amount present in the form of residual apatite minerals. The soda-insoluble fraction is, however, probably composed mainly of such minerals, and from the figures given in Table II amounts, in acid soils, to about 10 per cent of the total soil phosphorus. Some of this soda-insoluble phosphorus may be present as suggested, by Marshall(7), as an integral part of the clay crystal lattice, or it may be present as an insoluble titanium phosphate. The writer has not had the opportunity of investigating the solubility of the latter form of phosphate. In general, the following types of phosphorus compounds may be present in soils: (1) iron and aluminium phosphates, (2) organic phosphorus compounds, (3) phosphorus forming part of the clay colloidal complex, and phosphorus in combination with calcium either as (4) apatite or (5) some other form of calcium phosphate. Soils containing free calcium carbonate are likely to contain considerable amounts of types (4) and (5), type (5) probably tending to change to the more stable type (4). Any non-apatite calcium phosphate in acid soils will be soluble in sodium hydroxide. At present it is impossible to distinguish between the organic and inorganic forms of soil phosphorus, although there is some suggestion, as mentioned earlier by the writer, that boiling soil with sodium hydroxide solution in the presence of  $\text{CaCO}_3$  may provide a means of effecting at least a partial separation of the organic from the inorganic phosphorus. This point will

be the subject of further investigation. The results indicate that the organic phosphorus and the non-apatite calcium phosphates are brought into solution by sodium hydroxide. There remains the phosphorus in combination with sesquioxides and in the clay complex. The former, if present in the soil, is, from the results in Table III, completely soluble in sodium hydroxide solutions. Water-soluble phosphate when added to soils becomes fixed in an insoluble form. In acid soils this fixation of soluble phosphorus is generally believed to be brought about by the sesquioxides and the soil colloids. Its mechanism has been the subject of many investigations. A short review of this work has been given by Hibbard(6). It is clear from the work of Gaarder(5) and others that conditions occur in acid soils which can bring about the precipitation of the phosphate as iron and aluminium phosphate. It is also evident from the work of Mattson(8), Pugh(10), Demolon(3) and Ravikovitch(11) that the phosphate ion can exist in soil colloid complexes in a position where it is exchangeable by other anions. Scarseth(12) has recently put forward a theory to explain the mechanism of phosphate retention by aluminosilicate colloids under different conditions of saturation. According to this theory, the  $\text{PO}_4$  ion, in the H-clay, is dissociated from the complex and is in solution when iron is absent, but if iron ions are present, insoluble iron phosphate precipitates are formed; with the Na-clay at pH 6.0, a phospho-alumino-silicate is formed by the adsorption of  $\text{PO}_4$  on the colloidal surfaces by the aluminium bond; with Ca-clay at pH 6.0, the bivalent cation of the complex as well as aluminium of the complex absorbs the  $\text{PO}_4$ . This phosphorus would be exchangeable with the hydroxyl anion. Marshall accounts for the clay phosphorus as being present either as hydrated phosphate of iron or aluminium or, more probably, as forming part of the clay lattice—a phosphorus and aluminium atom replacing a silicon atom in the silicon layer of the crystal lattice. Thus it appears to be generally agreed that phosphorus is present in the soil colloidal complex but it seems impossible, at present, to distinguish between the iron and aluminium phosphates and the phosphorus taking part in anionic exchange which is a part of the colloidal complex itself. Both types of phosphate are soluble in sodium hydroxide and in view of the results given in Table II most of the inorganic phosphorus in acid soils must consist of one or other of these. There remains, however, the possibility, suggested by Marshall's theory, that some phosphorus may be present as a non-exchangeable integral part of the lattice. At present, there is no evidence to show how far the decomposition of the lattice proceeds under the attack of boiling sodium hydroxide solution. It is

therefore impossible to decide whether the phosphorus, if present in the form suggested by Marshall, is to be included in the soda-soluble category. If it is soda-insoluble, then the amount present in acid soils must be small. Thus, the evidence so far available shows that the only form of soil phosphorus that can be definitely placed in the soda-insoluble group is that possessing the apatite structure. Further work is necessary to decide whether some other type of clay phosphorus such as that suggested by Marshall is insoluble in sodium hydroxide.

Another possibility which should be explored is the effect of addition of soda-soluble and soda-insoluble phosphorus compounds to acid soils. A few preliminary experiments carried out by the writer with some of the acid soils mentioned in Table II show that shaking the soil for several days with finely ground apatite causes an increase in the amount of soda-soluble phosphorus, presumably through reaction between apatite and the soil. Under identical conditions apatite appears to react more readily with the soil than does the basic slag which was used in the experiments in Table III.

To sum up, the phosphorus compounds of the soil may be thus classified on the basis of their solubility in soda:

#### *Soluble*

Phosphorus in combination with sesquioxides.

Organic phosphorus.

Exchangeable phosphorus of clay complex.

Phosphorus of calcium compounds such as  $\text{CaHPO}_4$ .

Phosphorus of water soluble compounds.

#### *Insoluble*

Phosphorus in compounds of the apatite class.

#### *Doubtful*

Phosphorus in interior of clay lattice.

Phosphorus of titanium compounds.

#### SUMMARY

1. The solubility of the phosphorus of soils and of other phosphatic materials in solutions of sodium hydroxide has been investigated. A method for the extraction and determination of the soluble phosphorus is described.

2. With acid soils, especially when the exchangeable calcium has



been removed by leaching, an approximately constant amount of phosphorus is extracted by sodium hydroxide solution of strengths from 2 per cent to 20 per cent. The soda-soluble fraction amounts to 90 per cent of the total phosphorus.

3. Soils containing free calcium carbonate vary in the solubility of their phosphorus in sodium hydroxide solutions. Some behave similarly to acid soils, whilst others show only a small fraction of their total phosphorus to be soda-soluble. The extraction is complicated by the presence of calcium carbonate, which can precipitate from solution compounds which, in its absence, are soda-soluble.

4. From an examination of a number of phosphatic minerals and compounds, only basic slag and those known to possess an apatite structure have a very low soda-solubility.

5. The probable nature of the soda-soluble and soda-insoluble soil phosphorus compounds is discussed. The only type of compound that can be definitely placed in the soda-insoluble category is that having an apatite structure.

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# A STUDY OF THE CHEMICAL AND BACTERIOLOGICAL CHANGES OCCURRING IN GRASS SILAGE

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(With Four Text-figures)

ALTHOUGH silage has been made in this country for more than 50 years, the exact nature of the chemical and bacterial changes which occur during the period of ripening is still very imperfectly understood. There are several reasons for this.

(1) The chemical changes are compounded of those due to plant respiration and those brought about by bacterial growth, and since these take place to a large extent simultaneously, it is difficult to apportion the amount of the total change to each factor separately.

(2) There is good reason to believe that the extent and rapidity of the changes which occur in the body of the silo are influenced by such variable factors as the external temperature, the moisture content, botanical species and age of the crop, and the season of the year when cut.

(3) They are also dependent upon the method of packing at the time of ensiling, since this ensures more or less anaerobic conditions, and will therefore affect both the nature of the plant respiratory changes and the kind of bacterial flora which develops.

It follows that the data obtained by investigators with one kind of crop in one country cannot necessarily be taken as guides to the changes which will occur with a different crop or in another country with different climatic conditions.

That the micro-organisms in silage may, under some circumstances, be responsible for its complete deterioration, and that the product when subsequently fed to cattle may have ill effects, are well-known facts.

Annett & Russell<sup>(1)</sup> refer to such cases, and ascribe the effect to

## 272 *Chemical and Bacteriological Changes in Grass Silage*

decomposition products of the amine type which may occur under certain conditions. A similar case was noted at Jealott's Hill in 1929, when silage made from vetches and oats at a fairly immature stage of growth was fed to fattening cattle. The silage was of high moisture content and smelt strongly of butyric acid. The cattle lost weight and condition rapidly, and would not eat the silage freely.

Weimar<sup>(2)</sup> records three cases where feeding bad silage to cows was apparently responsible for the milk giving a positive reaction with the alcohol test. On discontinuing the feeding of the silage, the milk returned to normal, and this author concluded that, with some cows at any rate, poor silage will affect their physiological condition in such a way as to cause the milk to become abnormal.

These instances are sufficient evidence for the necessity of controlling the fermentation of silage in such a way as to prevent its deterioration. In order to accomplish this, the nature of the chemical changes occurring and the bacterial flora predominating in good silage must be understood, and the means studied whereby the most beneficial types of bacteria can be encouraged as a normal practice.

The chemical changes have been studied to some extent in different crops, while the bacteriology of maize silage has been investigated in America, and that of grass and clover mixtures in Germany. It appears, however, that comprehensive chemical data for the different stages of fermentation are lacking, and bacteriological results are even more meagre. Moreover, those crops which have been studied have usually been ensiled at a relatively advanced stage of maturity when there is a comparatively high carbohydrate and a low protein content. It is considered that the main problem in this country is to conserve material of a high protein and, hence, relatively low carbohydrate content, a condition which is ensured by using young grass as the crop.

For these reasons it was decided to investigate the changes which occur in silage made from young grass. Results of a study of the types of lactobacilli, the coliform bacteria and the obligate anaerobes in this type of silage have recently been published (Allen & Harrison<sup>(3,4,5)</sup>).

The experiments described here were designed, in the first place, to follow in detail the chemical and bacteriological changes which occur throughout the period of ripening of normal grass silage and, secondly, to find the effect upon such changes of the addition of various substances to the grass at the time of ensiling. The results obtained suggested a possible method of controlling the process of fermentation so as to minimize losses, and yield a ripe silage of maximum feeding value.

Where it was necessary to ensure uniform conditions or to study one variable factor while keeping others constant, small-scale laboratory experiments were designed. In other cases, samples were taken from silos in the field.

## EXPERIMENTAL

I. *The bacterial flora of fresh grass*

Since it may be assumed that the bacteria which attain large numbers in silage are initially present on the fresh grass, it is of importance to discover what types of bacteria make grass their constant habitat and what fluctuations in flora occur with differences of season and locality and with changes in weather. The numbers of micro-organisms of various kinds found in nine different samples of fresh grass are shown in Table I.

Table I. *Bacterial flora of different samples of fresh grass (numbers per gram)*

Sample	...	1	2	3	4	5
Mixed flora on aerobic plates at 30° C.		4,810,000	14,200,000	19,100,000	15,940,000	21,500,000
Thermophiles		—	—	—	—	—
Aerobic spore-formers		250	300	1,600	2,400	—
Coliforms giving presumptive test at 30° C.		1,000	10,000	1,000,000	100,000	—
Coliforms giving presumptive test at 37° C.		< 100	< 100	1,000	< 100	—
Lactobacilli		—	—	—	—	10,000,000
Spore-forming obligate anaerobes		10	100	10	100	—
Yeasts and moulds		1,220	25,300	10,400	2,090	—

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Sample	...	6	7	8	9
Mixed flora on aerobic plates at 30° C.		1,630,000	4,240,000	20,300,000	42,800,000
Thermophiles		—	—	2,070	1,600
Aerobic spore-formers		—	—	—	—
Coliforms giving presumptive test at 30° C.		100,000	1,000,000	10,000	1,000,000
Coliforms giving presumptive test at 37° C.		< 100	< 100	—	—
Lactobacilli		1,000,000	1,000,000	1,000,000	1,000,000
Spore-forming obligate anaerobes		10	100	10	100
Yeasts and moulds		—	—	—	—

Samples 1–4 were from closely situated areas, those from which 1 and 2 were taken having been ungrazed for 12 months previously, while those yielding 3 and 4 were grazed by sheep until 3 weeks before cutting. Samples 1 and 3 were exposed to the weather, 2 and 4 were covered for 3 weeks before cutting. Sample 5 was meadow grass cut in autumn,

## 274 *Chemical and Bacteriological Changes in Grass Silage*

samples 6 and 7 were similar grass cut in spring, and samples 8 and 9 were lawn-mowings from a bowling green in quite a different locality.

The flora isolated from Petri dishes incubated aerobically at 30° C. was very mixed, and comprised lactobacilli and coliforms with smaller numbers of spore-formers, micrococci, streptococci, yeasts, actinomyces and moulds. Bacteria which appear always to be present in large numbers on the surface of grass are lactobacilli (between 1,000,000 and 10,000,000 per g.) and coliforms (between 1000 and 1,000,000 per g.). The lactobacilli appeared to be almost entirely restricted to strains of *Streptobacterium plantarum*—characterized by comparative inactivity in plain litmus milk, but much stimulated in growth by the addition of yeast extract. The predominant coliform bacterium was always found to be a capsulated species, growing rapidly at 20–30° C., but unable when freshly isolated to grow at 37° C., to which the name *Bacillus (Aerobacter) aerogenes graminis* has been given (3).

It may be seen that obligate anaerobes, due no doubt to the unsuitable aerobic conditions obtaining on grass surfaces, are present in only small numbers.

### II. *Changes occurring in grass stored in tins at room temperature.*

#### *Influence of the addition of formalin*

Small-scale experimental silos were prepared by using heavy tins of 14 in. diameter. A layer of sand was placed at the bottom, and 25 lb. of fresh-cut grass packed in each tin, the grass being covered with a layer of sterile cotton-wool and weighted with a 3 in. layer of sand supporting a concrete block. In one series of tins, grass treated with 1 per cent formaldehyde was used, and in the other fresh grass mixed before packing with an equivalent quantity of water. The tins were stored at room temperature, which was low throughout the experiment, and fluctuated from about 10 to 15° C. At daily intervals for the first 5 days, and then after the eighth, eleventh and fifteenth days, the contents of a whole tin in each series were used for a bacteriological and a chemical examination.

Portions of the silage were abstracted with sterile tongs from different depths, combined and mixed. In each case 50 g. were used for making extracts and dilutions in sterile saline for bacteriological tests, and the remainder was submitted to chemical analysis. Dilutions of the untreated grass silage were plated on dextrose-bean-extract agar, and inoculated into yeast-extract-dextrose broth.

The bacterial counts are shown in Table II, the chemical data in Table III.

It may be seen that in neither type of silage was there any appreciable chemical change throughout the period of 15 days. The formalinized grass retained its green colour completely, and the colour of the untreated grass was only slightly brown. Moreover, the bacterial counts in the latter type of silage (except those for the first day, which seem to be anomalous) do not show a significant increase for the first 8 days. The flora was mixed, and consisted of lactobacilli, coliforms, yeasts, micrococci and spore-forming bacilli. No detailed investigations were made except in the case of the coliforms, which increased in numbers from about 200,000 per g. in the fresh grass to 80,000,000 per g. after 15 days' storage, and proved to consist almost exclusively of *B. aerogenes graminis*.

Table II. *Counts of micro-organisms per gram in silage made from (a) untreated, (b) formalinized grass, stored at room temperature in large tins*

Age in days	Untreated grass		Formalinized grass.
	Dextrose bean agar	Yeast-extract-dextrose broth	Yeast-extract-dextrose broth
0	21,500,000	10,000,000	—
1	92,000,000	1,000,000,000	—
2	8,100,000	10,000,000	—
3	22,100,000	100,000,000	1,000,000
4	21,200,000	10,000,000	1,000,000
5	11,600,000	100,000,000	100,000
8	167,000,000	100,000,000	1,000,000
11	258,000,000	1,000,000,000	10,000,000
15	850,000,000	100,000,000	100,000,000

Table III. *Chemical characteristics of silage made from (a) untreated, (b) formalinized grass*

	Original grass	Age in days						
		1	2	3	4	8	11	15
(a) Untreated:								
Total acidity c.c. N/10 acid	97.0	87.2	77.4	77.5	87.0	106.6	96.9	174.2
Volatile bases c.c. N/10 alkali	3.8	1.9	1.9	3.9	1.9	7.8	3.9	34.8
Volatile acids c.c. N/10 acid	19.4	17.4	13.5	17.4	19.3	25.2	13.6	13.5
(b) Formalinized:								
Total acidity c.c. N/10 acid	97.0	77.3	—	—	77.2	—	—	96.6
Volatile bases c.c. N/10 alkali	3.8	1.9	—	—	3.9	—	—	3.9
Volatile acids c.c. N/10 acid	19.4	9.7	—	—	19.3	—	—	25.1

These experiments appear to indicate that if the external temperature is sufficiently low, the changes due to plant respiration are extremely small and bacterial multiplication is very slow. It must be realized, of course, that the grass containers were comparatively small and their contents would rather easily acquire atmospheric temperature. In a silo

## 276 *Chemical and Bacteriological Changes in Grass Silage*

in a field the large bulk of material affords a measure of heat insulation, so that the central portion of the mass may retain a good deal of the heat brought about by its own respiration, thus *ipso facto* tending to raise its temperature to the point where respiration and bacterial growth proceed more quickly. Nevertheless, the lower the external temperature, the less quickly is this likely to occur.

### III. *The chemical and bacteriological changes occurring in grass stored in large test-tubes under controlled conditions. Influence of moisture content*

Chemical and bacteriological analyses of samples taken from large silos in the field are subject to errors due to adventitious circumstances and the difficulty of obtaining portions representative of the whole bulk. Thus fluctuations in external temperature during the period of trial, inequality of drainage in different parts of the same silo or in two different silos which are being compared, differences in moisture content of the grass packed in different silos, and the fact that the material at the bottom of the container is inevitably under greater pressure than that in the middle or near the top, thus creating differences in degree of anaerobiosis, are some of the factors which limit any attempts to find the influence of one particular set of conditions while keeping others constant.

For these reasons it was decided as a preliminary to follow the changes occurring in a series of small containers all packed with the same quantity of finely cut grass taken from a well-mixed bulk and stored at an even temperature. Conditions in a silo were simulated as far as possible by adopting the following methods:

The containers were large test-tubes measuring  $6 \times 1$  in. with a  $\frac{1}{2}$  in. layer of sand at the bottom. Fresh, finely cut lawn-mowings were thoroughly mixed, and 25 g. tightly packed into each container with the aid of a glass rod. A shallow layer of sand completed the packing, and the tube was fitted with a tight cork, as shown in Fig. 1. Molten pitch was poured over the top of the cork and allowed to flow round the rim of the tube, so as to seal against the air. When the pitch had set, a pin was plunged vertically through the pitch and cork, thus making a narrow hole, which allowed exit for evolved gases.

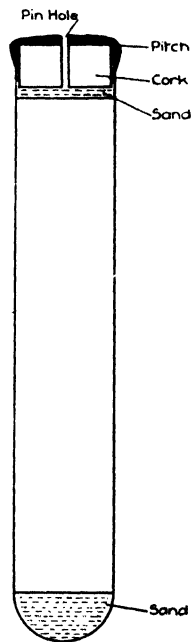


Fig. 1

Table IV. *Grass stored in tubes at 25° C. Summary of counts on various media at intervals during storage. Bacteria per gram of silage*

Age of silage days	Yeast dextrose agar at 55° C.		Yeast dextrose agar at 30° C.		Gas-formers in dextrose		Obligate spore- forming anaerobes		Lactobacilli	
	I	II	I	II	I	II	I	II	I	II
0	2,070	1,600	Grass alone		—	10 <sup>6</sup>	10	10 <sup>2</sup>	10 <sup>6</sup>	10 <sup>6</sup>
1	1,840	2,250	20,300,000	42,800,000	10 <sup>7</sup>	10 <sup>6</sup>	10	—	10 <sup>9</sup>	10 <sup>8</sup>
2	320	750	169,000,000	243,000,000	10 <sup>6</sup>	10 <sup>7</sup>	10	10	10 <sup>8</sup>	10 <sup>9</sup>
3	1,020	830	800,000,000	820,000,000	10 <sup>7</sup>	10 <sup>8</sup>	10	10	10 <sup>8</sup>	10 <sup>9</sup>
4	2,070	1,930	770,000,000	960,000,000	10 <sup>7</sup>	10 <sup>7</sup>	10	10 <sup>2</sup>	10 <sup>8</sup>	10 <sup>9</sup>
5	4,700	1,350	1,100,000,000	790,000,000	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>2</sup>	10	10 <sup>10</sup>	10 <sup>9</sup>
8	1,200	890	850,000,000	790,000,000	10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>8</sup>	10 <sup>8</sup>
11	3,690	1,630	520,000,000	650,000,000	10 <sup>9</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>9</sup>	10 <sup>9</sup>
15	10,000	8,400	414,000,000	493,000,000	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>9</sup>	10 <sup>8</sup>
24	23,600	18,300	890,000,000	195,000,000	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>9</sup>	10 <sup>8</sup>
35	9,000	10,000	265,000,000	219,000,000	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>9</sup>
45	8,500	11,400	84,000,000	165,000,000	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>8</sup>
			290,000,000	213,000,000	10 <sup>9</sup>	10 <sup>8</sup>				
			Grass with 10% added moisture							
0	2,070	1,600	20,300,000	42,800,000	—	10 <sup>6</sup>	10	10 <sup>2</sup>	10 <sup>6</sup>	10 <sup>6</sup>
1	700	870	720,000,000	253,000,000	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>2</sup>	10	10 <sup>9</sup>	10 <sup>9</sup>
2	2,600	970	740,000,000	690,000,000	10 <sup>4</sup>	10 <sup>6</sup>	10	10 <sup>2</sup>	10 <sup>9</sup>	10 <sup>8</sup>
3	1,230	1,440	760,000,000	600,000,000	10 <sup>7</sup>	10 <sup>7</sup>	10	—	10 <sup>9</sup>	10 <sup>8</sup>
4	1,170	1,220	890,000,000	740,000,000	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>9</sup>	10 <sup>8</sup>
5	1,010	910	980,000,000	830,000,000	10 <sup>9</sup>	—	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>9</sup>	10 <sup>8</sup>
8	5,410	4,480	800,000,000	270,000,000	10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>9</sup>	10 <sup>9</sup>
11	2,740	16,300	401,000,000	414,000,000	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>8</sup>	10 <sup>9</sup>
15	23,100	12,400	492,000,000	527,000,000	10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>9</sup>	10 <sup>8</sup>
24	2,140	2,470	144,000,000	407,000,000	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>9</sup>	10 <sup>8</sup>
35	7,600	6,200	132,000,000	69,000,000	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>9</sup>	10 <sup>8</sup>
45	1,070	1,500	179,000,000	177,000,000	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>8</sup>	10 <sup>8</sup>



## 278 *Chemical and Bacteriological Changes in Grass Silage*

Two series of tubes were packed in this way, one with untreated grass, the moisture content of which was found to be 75.1 per cent, and the other with the same grass containing 10 per cent additional water, added by means of a graduated pipette to the contents of each tube before packing. The tubes were incubated at 25° C., and the whole contents of two duplicate tubes of each series used for chemical and bacteriological investigations at intervals throughout a period of 45 days. The technique adopted was as follows:

*Bacteriological examination.* The contents of each tube were emptied into a sterile covered mortar, well ground with sterile sand and macerated with 225 c.c. of sterile water at 45° C. for 15 min. This was regarded as a 1/10 dilution, except in the case of the grass to which 10 per cent moisture had been added, for which a small correction was applied later. Further dilutions were prepared in tubes of saline, and 1 c.c. of each dilution was inoculated into the following media, which were incubated at 30° C., except where otherwise stated:

- (1) Yeast-extract-dextrose agar plates.
- (2) Yeast-extract-dextrose-agar plates incubated at 55° C.
- (3) Yeast-extract-litmus-dextrose-peptone broth, in tubes containing Durham's tubes.
- (4) Robertson's cooked meat, pasteurized at 80° C., after inoculation and incubated anaerobically.
- (5) Yeast-extract-dextrose broth, not pasteurized, incubated anaerobically.

Medium 1 showed the number of micro-organisms growing aerobically at 30° C., medium 2 the count of thermophilic aerobes, medium 3 the number of organisms forming gas from dextrose, medium 4 the number of spores of obligate anaerobes, and medium 5 had been previously found to be the best for lactobacilli (4). The duplicate counts obtained in these media at intervals during the course of the investigation are shown in Table IV.

*Chemical examination.* The aqueous extract remaining from the bacteriological examination was decanted from the residue of grass and was centrifuged to clarify it, the clear supernatant fluid being used for determination of the following:

- (1) Titratable acidity, using standard baryta and phenolphthalein.
- (2) Amino nitrogen by an alcohol-formalin titration.
- (3) pH by means of the quinhydrone electrode.
- (4) Volatile acids by steam-distilling 50 or 75 c.c. of the extract at

constant volume and titrating successive equal portions of the distillate until a small constant value was obtained.

(5) Lactic acid by the method of Friedemann & Graeser (6).

The last two values were not estimated at every stage of the investigation.

Results of these determinations for duplicate tubes, both for silage made from normal grass and that made from grass with added moisture, are shown in Table V.

Table V. *Grass stored in tubes at 25° C. Summary of values for total acidity and amino N (expressed as c.c. N/10 per 100 g. dry matter) and pH*

Age of silage days	Grass alone						Grass with 10% added moisture					
	1			2			1			2		
	pH	Total acidity	Amino N	pH	Total acidity	Amino N	pH	Total acidity	Amino N	pH	Total acidity	Amino N
Fresh grass	6.59	227	247	6.59	227	247	6.59	227	247	6.59	227	247
1	5.75	—	—	5.25	—	—	4.78	435	464	5.01	410	512
2	4.35	685	662	4.63	563	578	4.54	586	517	4.58	581	622
3	4.54	626	677	4.56	608	685	4.42	668	656	4.46	607	653
4	4.39	636	715	4.44	636	733	4.39	712	671	4.47	689	694
5	4.23	758	735	4.37	710	735	4.40	694	750	4.59	822	671
8	4.52	783	824	4.56	687	829	4.25	750	784	4.14	799	771
11	4.30	730	844	4.39	715	842	—	—	—	4.44	822	899
15	4.21	740	892	4.30	690	867	4.11	843	809	4.18	835	901
24	4.25	715	908	4.65	679	941	3.86	802	900	3.86	817	876
35	3.97	702	748	4.70	497	880	3.99	850	866	4.00	891	899
45	4.85	521	1115	4.58	606	1182	4.63	469	1068	4.71	488	1039

*Values for lactic acid and volatile acids as c.c. N/10*

Days old	1		2		1		2	
	Lactic acid	Volatile acids	Lactic acid	Volatile acids	Lactic acid	Volatile acids	Lactic acid	Volatile acids
Fresh grass	—	49	—	—	—	—	—	—
24	561	—	508	—	591	145*	587	—
35	414	234*	420	—	622	185*	627	—
45	304	279*	326	—	377	272*	339	—

\* Combined figures for Series 1 and 2.

*Discussion of results*

The chemical data and bacterial counts show that, with few exceptions, there is reasonable consistency between duplicates, indicating that the method of packing and incubating the silage has largely eliminated inconsistencies which might otherwise occur through fluctuation in uncontrolled factors. Moreover, since bacteriological and chemical tests were made in each case on the same extract, there is less likelihood of error in attempts to correlate the two.

In general, it may be seen that in both series of tubes the pH fell rapidly during the first 2 days, and thereafter showed comparatively little change except for temporary fluctuations. Correlated with this is

## 280 *Chemical and Bacteriological Changes in Grass Silage*

an initial sharp rise in titratable acidity, after which this value remained high until near the end of the experiment. It should be mentioned here that in the last stages of the incubation period, the tubes showed considerable mould growth near the surface, which probably affected the values for titratable acidity and lactic acid.

Of the separate groups of bacteria counted, it appears that the thermophiles never reached high numbers. This no doubt was due to the effect of incubating small containers at a constant temperature of 25° C. In a large silo the inner portions acquire an appreciably higher temperature than the outside air, and thermophiles develop profusely at a certain stage. This is shown in a later experiment.

The gas-formers in dextrose broth were found to be coliforms. It has been previously pointed out (3) that the species of coliform predominant on grass may appear in moderate numbers in the early stages of silage fermentation, but that it does not normally attain large numbers owing to the rise in temperature of the silage inhibiting its growth. In these test-tube experiments, the maintenance of a moderate temperature allowed its continued growth. In fact, the coliforms increased in the first 3 or 4 days from 1,000,000 to approximately 100,000,000 per g.

The lactobacilli, present in the fresh grass to the extent of 1,000,000 per g., rapidly increased in 24 hours to approximately 1,000,000,000 per g. and remained at this high level throughout.

Both the last two groups of bacteria produce lactic acid from carbohydrates, the lactobacilli of the *Streptobacterium plantarum* type (shown in a previous communication (4) to be the predominant type in grass silage) forming only comparatively small amounts of other metabolic products. The figures for lactic acid in Table V show that, after 24 days (the first occasion on which this product was estimated) lactic acid represents the major part of the titratable acidity in all four tubes of silage. Thereafter the silage made from the grass alone shows a decrease in lactic acid (although it still remains an appreciable proportion of the total acidity), while that from the moist grass shows a higher value after 36 days and a sudden fall subsequently. Although, in the absence of precise knowledge as to the products of plant respiration, it would be unwise to make a definite statement, it seems reasonable to assume that this lactic acid is largely, if not entirely, brought about by the growth of lactobacilli and coliform bacteria, particularly the former. The decrease in lactic acid towards the close of the experiment indicates that this substance is being decomposed, either by other groups of bacteria or by the mould which was in evidence at this stage. A similar effect was noted by Amos & Woodman (7) working with oats and tares.

The lactobacilli common in grass silage form small quantities of volatile acid (almost entirely acetic) but this applies to other bacteria also. In the view of some observers anaerobic plant respiration results in the formation of a certain amount of volatile acid. The values for this substance shown in Table V, therefore, doubtless represent the combined

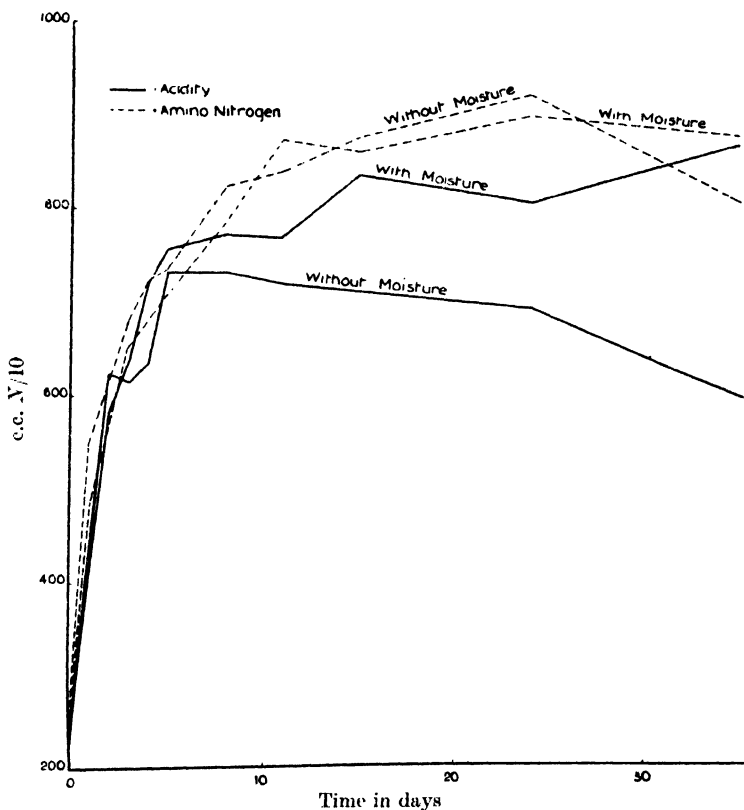


Fig. 2. Showing rate of increase in titratable acidity and in amino nitrogen in test-tube silage made from (a) grass alone, (b) grass with 10 % added moisture.

effect of several factors. In the later stages of silage fermentation it is quite certain that obligate anaerobes produce appreciable quantities of volatile acids, especially butyric.

Growth of the spore-forming anaerobes to considerable numbers occurred after the eighth day and these were still increasing at the end of the experiment. Two factors probably control their growth—degree of anaerobiosis and *pH*. The initial lag is undoubtedly due to the time taken

## 282 *Chemical and Bacteriological Changes in Grass Silage*

for plant respiration and the growth of other types of bacteria to use up the oxygen originally present in the container and to create suitable anaerobic conditions. If the *pH* is sufficiently low the anaerobes will not develop even then, but some types are evidently not restricted by *pH* values between 4.0 and 4.5. In the later stages the aerobic growth of

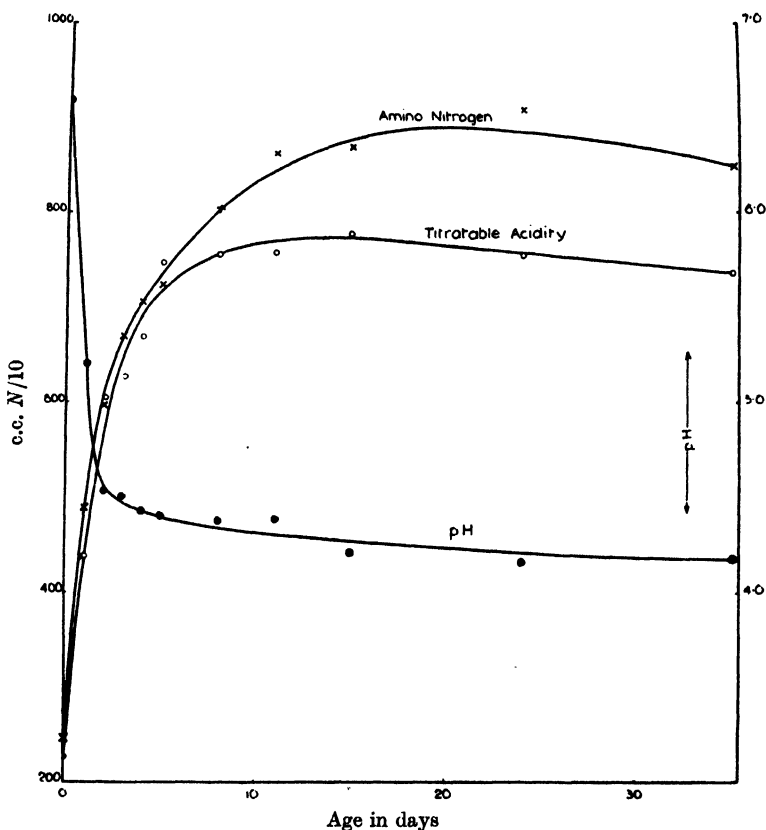


Fig. 3. Curves showing general tendency of chemical changes in test-tube silage.

mould at the surface helps to maintain anaerobic conditions in the lower layers of the silage. The predominant species of anaerobe in this series of experiments was found to be *Clostridium sporogenes*.

The general course of the chemical changes occurring in these tube silages is shown graphically in Fig. 2, the curves in each case giving the average value for the two duplicates in each series. It appears that addition of water to the grass made no significant difference to the

subsequent proteolysis in the silage, but that there is a somewhat greater development of acidity in the more moist silage. Since the bacterial counts do not show a significant difference in the two types of silage it is difficult to assign a reason for this. No accurate counts of moulds were made, but it is possible that mould growth may have occurred to a somewhat greater extent in the drier silage, owing to its rather less compact nature, and reduced the titratable acidity slightly by using some of the lactic acid as a source of carbon.

Although it is evident that in this particular case the addition of moisture had no effect on bacterial growth it cannot be assumed that this would hold in all cases. Presumably a critical point exists at which the moisture content of the grass is so low that bacterial growth is retarded. It was, in fact, observed by Dalla Torre<sup>(8)</sup> that the moisture content of grass silage was a very important factor affecting the numbers of bacteria found.

The general nature of the changes taking place for the first 35 days in tube silage is shown in Fig. 3, where each point on the curves represents the average of the results for four tubes, two without and two with moisture. The values for the final determinations are not given as it was concluded that the growth of mould had produced changes which were not typical.

#### IV. *The chemical and bacteriological changes occurring in silage stored in small concrete silos*

The object of this experiment was to trace the course of the chemical changes and development of bacterial flora in normal grass silage under field conditions. By this means it was expected to obtain data for the typical course of decomposition of the grass proteins and carbohydrates, and for the numbers of the various groups of micro-organisms concerned, and to isolate and identify the predominant species at various stages. By combining these data with those obtained in the small-scale experiments previously described a standard was secured with which to compare similar figures obtained in future experiments.

Twelve small, circular, concrete silos, 5 ft. in diameter and 3 ft. in height, each with a capacity of some 10 cwt., were all filled in 1 day with freshly cut young spring grass. Care was taken to apply the same pressure to each one and undue contamination from external sources was avoided by the use of freshly washed boots when treading the silage and the substitution of a sheet of stout, paper-lined hessian between the soil covering and the upper layer of the silage. One silo was opened on each

## 284 *Chemical and Bacteriological Changes in Grass Silage*

of the following days after filling: 1, 2, 3, 4, 5, 8, 11, 17, 28, 43, 87, 117, and the contents used for a chemical and bacteriological analysis.

*Bacteriological investigation.* Portions of the silage were abstracted with sterile tongs from different depths and combined to make a total sample of about 1 kg. This was done for the top and bottom halves of the silo separately in each case. After cutting each sample with sterile scissors into short lengths and mixing them, 50 g. were extracted in a sterile mortar with 450 c.c. of sterile saline (45° C.) and dilutions prepared in tubes of saline from this extract with which to inoculate various media.

The media used, the main types of bacteria isolated from each and their numbers per gram of silage at different stages are shown in Table VI. The nature of the flora developing in each medium was ascertained by making stained preparations and isolating and identifying predominant species at each stage.

*Nature of bacterial flora.* The *first group* in Table VI consists of bacteria capable of growing aerobically on yeast-extract-dextrose-agar plates at 30° C. The numbers remained more or less constant for the first few days, increased rather rapidly between the fourth and eighth days and remained high until about the fourth week, after which they declined. As might be expected, the flora of the fresh grass was very mixed, consisting of streptococci, micrococci, coliform bacteria, spore-formers, lactobacilli and yeasts. The increase in numbers at the peak shown in Table VI was found to be due almost entirely to spore-formers of the *B. subtilis* and *B. megatherium* type and to lactobacilli.

*Group II* consists of thermophilic organisms capable of growing aerobically on yeast-extract-dextrose-agar at 55° C. In the fresh grass there were very few and the numbers in the silage were quite small for the first few days. They showed a sudden large increase in growth between the eleventh and seventeenth days and then declined. The flora here was not mixed but confined to one type. It consisted of Gram-positive rods, none of which showed spore formation at 55° C. They were capable of growth at 30 and 37° C. and most of them formed spores at these temperatures although some cultures did not. They all proved to be strains of *B. subtilis*, some of them apparently being asporogenous variants. It is worthy of note that the increase in thermophiles corresponded with a rise in the temperature of the silage (see p. 288).

The *third group* consists of coliform bacteria—by which is meant those organisms giving a positive presumptive test in lactose-bile-salt broth. Parallel tests were made throughout the investigation at incubation temperatures of 30 and 37° C. and it may be seen that the numbers

Table VI. *Normal grass silage in small concrete silos. Numbers of bacteria per gram of silage at different ages*

Types of bacteria ...		Aerobes		Thermophilic aerobes		Coliforms		Obligate anaerobes		Lactobacilli			
Medium used	Age (days)	Yeast dextrose agar		Yeast dextrose agar		Bile-salt lactose broth		Yeast dextrose broth*		Yeast dextrose broth		Yeast dextrose agar	
Incubated at		30° C.		55° C.		30° C.		37° C.		30° C. anaerobically		30° C.	
		Top half	Bottom half	Top half	Bottom half	Top half	Bottom half	Top half	Bottom half	Top half	Bottom half	Top half	Bottom half
Fresh grass	1	2,930,000	2,930,000	—	—	10 <sup>8</sup>	10 <sup>8</sup>	<10 <sup>2</sup>	<10 <sup>2</sup>	10	10	10 <sup>8</sup>	10
	2	8,700,000	5,750,000	540	<10	10 <sup>8</sup>	10 <sup>8</sup>	<10 <sup>2</sup>	<10 <sup>2</sup>	10	10	10 <sup>8</sup>	<10
	3	7,900,000	2,230,000	370	1,020	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10	10	10 <sup>8</sup>	10
	4	19,300,000	5,610,000	38,100	600	10 <sup>8</sup>	10 <sup>8</sup>	<10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>8</sup>	300
	5	3,380,000	20,800,000	817,000	1,240	10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>7</sup>	2,400
	6	3,780,000	28,400,000	11,000	610	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>7</sup>	3,500
	8	6,900,000	125,000,000	270,000	3,080	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>8</sup>	840,000
	11	1,080,000	55,000,000	274,000	54,000,000	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>8</sup>	210,000
	17	1,050,000	81,300,000	350,000	116,000,000	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>8</sup>	520,000
	28	3,170,000	114,000,000	101,000	192,800	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>7</sup>	190,000
	43	10,400,000	10,900,000	28,800	37,680	<10 <sup>2</sup>	<10 <sup>2</sup>	10	10	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>7</sup>	104,000
	87	6,600,000	3,080,000	25,800	12,380	10	10	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>8</sup>	5,000,000
	117	—	380,000	—	21,800	—	10 <sup>2</sup>	—	10 <sup>2</sup>	—	—	—	350,000



## 286 *Chemical and Bacteriological Changes in Grass Silage*

growing at the lower temperature are much higher. Previous investigations<sup>(3)</sup> had shown that the predominant coliform organism in grass (often present to the extent of 1,000,000 per g.) was a capsulated species, forming acetyl-methyl carbinol from dextrose, methyl red positive, giving positive growth in Koser's citrate medium and very slowly liquefying gelatin. It was distinguished by the fact that when freshly isolated it grew rapidly at 30° C. but refused to grow at 37° C. It would therefore not be detected by the usual presumptive test. It was this organism (*B. aerogenes graminis*) which was responsible for the greater numbers shown in the 30° C. than in the 37° C. test in Table VI. The fact that it shows little or no increase in numbers in the silage is probably due to the rise in temperature of the silage inhibiting its growth.

The coliforms giving the positive presumptive test at 37° C. were found to consist of *B. coli*, *B. cloacae* and intermediate types.

*Group IV* consists of spore-forming obligate anaerobes. The media used for isolation, and to give an idea of the numbers present, were yeast-extract-dextrose broth and Robertson's cooked meat medium, the media being pasteurized at 80° C. for 20 min. after inoculation and before anaerobic incubation. It may be seen that the numbers showed a gradual increase as the silage aged, reaching a maximum of 1,000,000 per g. after about 3-4 weeks. These were, of course, the number of spores only, no attempt being made to count vegetative cells. Pure cultures of the predominant species were isolated on slopes of inspissated serum and their reactions studied with a view to identification. Throughout the whole period of fermentation only one type of anaerobe was isolated. This produced spores readily, showed both proteolytic and saccharolytic powers (forming acid and gas from dextrose and maltose) and haemolysed blood. Its characters corresponded with those of *Clostridium sporogenes*.

*Group V* consists of lactobacilli, of which a detailed account has been previously published<sup>(4)</sup>. The true numbers in the silage are given much more nearly by the yeast-extract-dextrose broth incubated anaerobically than by the shake cultures at pH 4.2 owing to the latter inhibiting the growth of all but the more aciduric cells. Nevertheless the latter are useful for isolation of pure cultures; 104 pure strains were isolated from the broth and shake cultures at different periods throughout the investigation and classified on the basis of reactions in litmus milk, yeast-extract-litmus milk, dextrose-litmus milk, and yeast-extract-dextrose-litmus milk, and in various sugar broths. The nature of the lactic and volatile acids formed from dextrose in the case of eight representative strains was determined. In this way it was found that the predominant strains of lactobacilli throughout the period of ripening

were of the *Streptobacterium plantarum* type, all stimulated (though to a varying extent) by yeast extract, and producing, either entirely or mainly, the inactive form of lactic acid, together with small quantities of acetic acid. They differed considerably in their range of carbohydrate fermentations. The increase in numbers of lactobacilli in this silage was not so rapid as in the test-tube experiments, but they were present to the extent of 1,000,000,000 per g. in the lower half of the silage after about 17 days.

One other group of bacteria should be mentioned—a type giving vigorous evolution of gas from carbohydrates, possessing somewhat unusual characters and which may have played some part in the fermentation of this particular silage. They were first detected in the lower dilutions of the shake cultures at pH 4.2 used to isolate the lactobacilli. These were completely broken up by gas formation after about 48 hours' incubation. A long, slender, motile, Gram-positive, non-sporing rod was isolated which fermented a large number of carbohydrates and higher alcohols—including dextrose, lactose, sucrose, maltose, mannite, raffinose, inulin, salicin, dextrin, xylose and starch—with abundant gas formation. Later a number of spore-forming bacteria were isolated from the yeast-extract-dextrose-agar plates which proved to have the same biochemical characters as the first group and one type appeared to be an asporogenous variant of the other. The spore-forming type was found in numbers between 5 and 10 millions per g. in the middle stages of the silage ripening, and from the fact that it actively fermented xylose, dextrin and starch and increased considerably in numbers as the silage aged it is reasonable to suppose that it contributed something to the general breakdown. The organisms show both proteolytic and saccharolytic properties, are facultative and can use ammonium salts as a source of nitrogen—being able to grow perfectly well anaerobically, for example, in a medium containing only dextrose and ammonium phosphate.

In general it may be said that the increase in bacterial numbers in this silage was less rapid than that which occurred in the test-tube silage—a difference which may be due to the fact that the grass used for the former was cut after a prolonged dry period and consequently had a lower moisture content at the time of ensiling—though the general nature of the flora (particularly as regards coliform bacteria, lactobacilli and obligate anaerobes) was similar. Two striking departures from this are the large number of aerobic spore-formers which appeared on the plates at 30° C. and the sudden increase in numbers of thermophilic bacteria between the eleventh and seventeenth days.

*Chemical investigation.* Owing to the small quantities of herbage

## 288 *Chemical and Bacteriological Changes in Grass Silage*

ensiled and the special care taken during filling and in sealing the silos, the final product was somewhat above average, and judging from the figures the fermentation was somewhat less than that of normal silage. The grass used had the following analysis:

Table VII. *Composition of grass used to make silage*

	Dry matter %
Ether extract	2.74
Fibre	23.10
Crude protein	10.00
N-free extractives	56.21
Ash	7.95
"True" protein	8.75
Calcium (CaO)	0.86
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	0.66
Dry matter in fresh grass	30.2

The protein content is not very high but the fibre content is surprisingly low and the dry-matter content is high. Despite this, the material packed well and gave silage of excellent quality. The temperature of the silage was taken whenever a silo was opened and at no time was this excessive.

This is shown by the following temperatures recorded by a thermometer inserted in the silage immediately after opening the silo:

Age of silage (days)	...	...	1	2	3	4	5	8	11	17	28	43
Temperature (°F.)	Near top		72	70	86	102	99	98	93	95	80	75
	Near bottom		68	68	68	78	82	80	82	92	75	79

The true temperatures attained by the silage before opening would probably be somewhat, though not much, higher than these.

Samples of silage were taken from the same material as that for bacteriological examination. They were fairly large and representative of the whole material in the first five silos and of the top and bottom half respectively in the silos opened subsequently. These samples were all chaffed and thoroughly mixed for analysis.

The method of Foreman (9) as modified by Woodman (10) was used to measure the total acidity, volatile bases, amino acids and volatile organic acids. This process depends on the addition of alcohol to an aqueous extract of the silage sample. The extract after addition of alcohol is titrated to give the total acidity. Another aliquot is steam-distilled, after the addition of alkali, to free the volatile bases which are titrated. An alkalinity develops in the distilling flask, which is titrated; it is called

Table VIII. *Total acidity, volatile acids, amino acids and volatile bases in ordinary grass silage*

Days old Fresh grass	Total acidity c.c. N/10 acid per 100 g. dry matter	Developed acidity c.c. N/10 acid per 100 g. dry matter	Volatile acid as acetic acid		Amino acids (stated as crude protein)		Volatile bases (stated as crude protein)		Crude protein in dry matter %	Dry matter %
			In fresh silage %	In dry matter %	In fresh silage %	In dry matter %	In fresh silage %	In dry matter %		
1	377	252	0.02	0.08	0.33	1.10	0.00	0.00	10.00	30.2
2	433	297	0.03	0.11	0.37	1.19	0.00	0.00	9.88	30.65
3	440	321	0.05	0.15	0.32	1.05	0.07	0.22	9.92	30.2
3	375	225	0.05	0.15	0.40	1.31	0.05	0.16	10.33	30.35
4	407	219	0.06	0.19	0.50	1.64	0.05	0.16	10.64	30.35
5	638	334	0.07	0.22	0.83	2.66	0.08	0.27	10.44	31.2
8 Top	947	578	0.25	0.83	0.96	3.20	0.15	0.51	10.81	30.1
11 Top	1037	695	0.39	1.24	0.93	2.99	0.23	0.75	10.93	31.1
Bottom	747	459	0.17	0.58	0.73	2.49	0.15	0.51	11.44	29.4
Bottom	663	424	0.16	0.56	0.60	2.09	0.18	0.64	11.51	28.7
Bottom	890	546	0.16	0.53	0.90	3.00	0.21	0.72	11.80	29.9
Bottom	737	443	0.15	0.52	0.73	2.57	0.20	0.70	10.23	28.5
Bottom	902	567	0.26	0.89	0.86	2.93	0.21	0.73	11.25	29.5
Bottom	926	595	0.27	0.92	0.83	2.89	0.32	1.10	12.31	28.8
Bottom	1021	640	0.36	1.27	0.93	3.34	0.20	0.72	—	28.0
Bottom	1256	873	0.50	1.75	0.97	3.35	0.25	0.87	—	28.8
Bottom	823	522	0.44	1.56	0.73	2.64	0.32	1.14	11.88	27.8
Bottom	856	556	0.50	1.88	0.70	2.62	0.23	0.87	12.69	26.8

## 290 *Chemical and Bacteriological Changes in Grass Silage*

"amino acids" by Foreman, and gives a useful relative measure of compounds of this type.

After acidification the volatile acids are distilled off and titrated. It is usual to calculate the values in terms of the content per 100 g. of fresh silage, but this is of little value for comparative purposes since the moisture content may vary very considerably even in the same silo.

The acidity which has developed in the silage can be estimated by subtracting the amino-acid acidity from the total acidity as measured by the Foreman method. This developed acidity corresponds to the titratable acidity of the laboratory experiments.

The results are tabulated in Table VIII.

The samples from the top and bottom of each silo do not show any great variation. The total acidity and volatile acids are somewhat higher in the bottom layer than the top, but the volatile bases do not show much change with depth and the amino acids are slightly higher in the upper layers. It is of interest to consider the volatile base and amino-acid N as a percentage of the total nitrogen. In Table VIII they have been given in terms of crude protein ( $N \times 6.25$ ) and the total nitrogen has been corrected for volatile bases lost on drying the sample on the basis that 50 per cent of the volatile bases would be lost during the drying process, a figure which our experience (Watson & Ferguson (11)) has shown to be approximately correct for silage containing like amounts of volatile bases. The figure was obtained by estimating the volatile bases on the fresh material and again on the sample after it had been dried. The percentage of the total nitrogen in the form of volatile-base and amino-acid N is given in Table IX.

Table IX. *Percentage of the total nitrogen in the form of volatile-base and amino-acid N*

Age of sample	Volatile-base N % of total nitrogen	Amino-acid N % of total nitrogen
0	0.00	11.00
1	0.00	12.00
2	2.20	10.54
3	1.59	12.73
4	1.54	15.44
5	2.55	25.47
8 Top	4.60	29.63
Bottom	6.82	27.34
11 Top	4.46	21.77
Bottom	5.54	18.17
17 Top	6.10	25.42
Bottom	6.86	25.14
28 Top	6.51	26.07
Bottom	8.12	23.49
87 Top	9.60	22.22
Bottom	6.87	20.63

The occurrence of apparent inconsistencies due to the individuality of each silo is well brought out by the figures for chemical analysis of the silo opened on the eighth day. Here the total acidity, volatile bases and volatile acids are all abnormally high, particularly in the lower layers of the silo. It is, perhaps, significant that the numbers of aerobic spore-formers, coliforms and lactobacilli for the same silo are collectively higher than those in the preceding or succeeding silo.

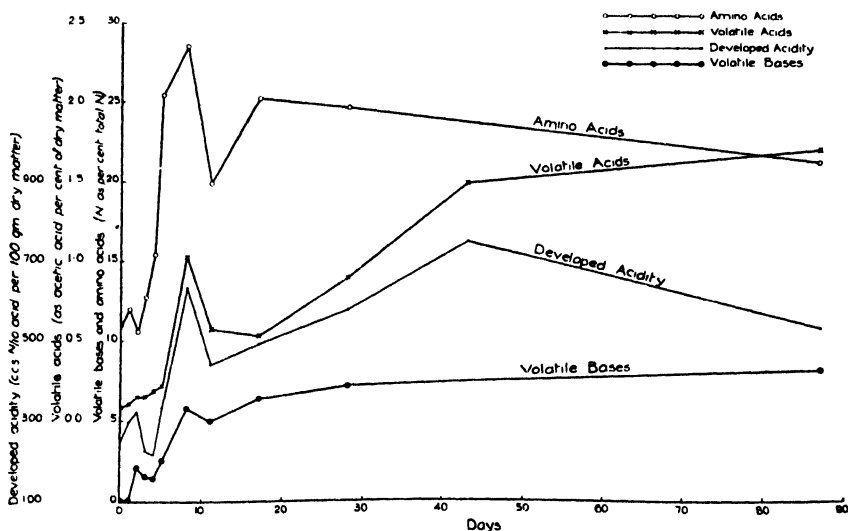


Fig. 4. Curves showing general tendency of chemical changes in silage made in small concrete silos.

The curves in Fig. 4 show the main data obtained in the examination of the samples of silage. For the latter part of the investigation the figures for top and bottom layers of the silos have been averaged. The developed acidity is plotted in terms of c.c. of  $N/10$  acid per 100 g. of dry matter, the volatile acids as g. acetic acid per 100 g. dry matter. The volatile bases and amino acids have been plotted in terms of the percentage of the total nitrogen which they have supplied at the different dates.

The total acidity remained fairly constant for the first 5 days and rose fairly rapidly thereafter by the eighth day after which it kept up until the forty-third day. The individual variation in the silos is probably responsible for the fall on the eleventh day or alternatively the values on the eighth day may have been too high. There seems to have been a tendency for the acidity to fall by the eighty-seventh day.

## 292 *Chemical and Bacteriological Changes in Grass Silage*

The volatile acids did not alter appreciably in the first 5 days but rose fairly rapidly thereafter to the forty-third day and were still higher on the eighty-seventh day, though the rate of increase during this latter period was much less than between the fifth and forty-third days. Neither total acidity nor volatile acids show a high value until the eighth day.

The percentage of total nitrogen in the form of amino acids began to rise on the third and fourth days and was at its maximum on the eighth day. After this the percentage of amino N tended to fall slightly.

During the first 5 days there was only a slight formation of volatile bases, but the percentage of volatile-base N increased perceptibly on the eighth day and increased only slightly thereafter.

The values for percentage of volatile-base N were relatively low throughout and show that the changes in the silo had been controlled satisfactorily, the breakdown of the protein not being permitted to proceed to such a stage that large amounts of volatile bases were formed. The percentage of the total nitrogen present as volatile bases is a useful index of the quality of the silage and judged by this criterion the silage would be classed as good.

It is apparent that on plotting the figures for any particular chemical change the points lie much less close to the general curve than was found in the case of the test-tube silage. This is no doubt partly due to the impossibility of standardizing all the variable factors in different silos even when filled from the same grass on the same day and apparently under the same conditions, and partly to the difficulty of securing a sample for analysis which is truly representative of the whole bulk of material in the silo. The major part of all the chemical changes in these silos appears to have occurred during the first 30 days.

### CONCLUSIONS

1. The chemical changes associated with the ripening of grass silage result in a rapid development of acidity which lowers the pH from approximately 6.5 to between 5.0 and 3.5. Simultaneously, proteolysis occurs, but is normally checked by the increasing acidity. In normal silage the acidity is accounted for almost entirely by lactic and acetic acids, butyric acid being characteristic of silage of poor quality. The rapidity with which these changes take place varies with conditions such as external temperature and method of packing, but usually the main changes occur in the first 10 days.

2. Coliform bacteria and lactobacilli appear to form the main bulk of the flora characteristic of fresh grass and they are therefore present in

large numbers at the commencement of the silage ripening. The coliforms are almost entirely of a type which will not grow at temperatures much above 30° C. and therefore their growth in silage is limited to the very early stages before the temperature of the mass has risen to any extent. The lactobacilli increase considerably in numbers in the early stages and are not inhibited by the rising temperature. These two groups of organisms—and particularly the lactobacilli—are considered to be mainly responsible for the formation of the lactic and acetic acids.

Obligate anaerobes are present in only very small numbers on fresh grass and do not begin to develop in the silage until several days after ensiling, when conditions have become suitably anaerobic. The extent of their proliferation depends on the availability of residual carbohydrate and protein and the restraining effect of pH.

3. The miscellaneous flora of fresh grass, which includes micrococci, yeasts and aerobic spore-formers, undoubtedly contributes to the general chemical breakdown in the silage, normally only to a limited extent. The influence of streptococci appears to be negligible and yeasts, though sometimes present in larger numbers, do not persist for long, but spore-formers, particularly thermophilic strains, develop considerably at certain stages, depending on availability of oxygen and the temperature of the silage.

4. Production of good silage appears to depend largely on the early conversion of the available carbohydrate to lactic acid, so that in the later stages of ripening the obligate anaerobes find supplies of carbohydrate restricted and the pH unfavourable for development. Destructive saccharolysis and proteolysis are then not extensive.

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# THE EFFECT OF THE ADDITION OF VARIOUS MATERIALS AND BACTERIAL CULTURES TO GRASS SILAGE AT THE TIME OF MAKING ON THE SUBSEQUENT BACTERIAL AND CHEMICAL CHANGES

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THE investigations previously described<sup>(1)</sup> have demonstrated that the changes occurring in the early stages of silage fermentation result in simultaneous development of acidity and degradation of proteins. There seems little reason to doubt that the lactobacilli play an effective part in the former by virtue of their formation of lactic acid. Contributions to proteolysis are undoubtedly made by various groups of bacteria, and in the later stages of ripening the spore-forming anaerobes, if numerous, will cause extensive protein breakdown and formation of volatile acids, especially butyric acid. The growth of the latter group of bacteria is greatly affected by the *pH* of the silage, for at values below about 4.0 the only group capable of anything like active multiplication is the lactobacilli. In fact the A.I.V. method of making silage consists in the addition of mineral acids at the commencement in quantities sufficient to reduce the *pH* to approximately 3.5. The production of good silage with a minimum loss of feeding value, low proteolysis and the presence of only small quantities of butyric acid may therefore be regarded as depending on the formation of sufficient acid in the earlier stages to retard the activities of the obligate anaerobes and proteolytic bacteria in the later stages.

The difficulty of making good silage from young grass of high protein content appears to be due to the comparative lack of readily fermentable carbohydrates to stimulate the formation of lactic acid in order to keep the other fermentations in subjection.

It was in order to see to what extent the addition of carbohydrate

during ensiling influenced the course of the subsequent changes in the silage that the following experiments were devised. Moreover, since the influence of lactobacilli is important and beneficial results have been claimed by Peterson, Fred and co-workers<sup>(2,3,4)</sup> and by Remm & Weiske<sup>(5)</sup> as a result of inoculation of silage (made from various crops) with species of these, it was decided to test the effect of adding cultures of lactobacilli when the grass was being ensiled.

The silos used in the experiment were made of concrete, 5 ft. in diameter and 3 ft. 6 in. deep, fitted with a movable steel over-silo of like size. All the silos were filled on 1 June 1933 with grass cut from one area, and conditions were kept the same during the filling of each silo. The grass used was of moderate quality, 8-10 in. long, but fairly leafy, and a digestibility trial was carried out on the material at the time of cutting. Sheep were used for the test, which extended for a week before and after the actual date of filling. During the filling of the silos, the material was carefully sampled for dry-matter determination and subsequent analysis.

At intervals over a period of 2 months samples were abstracted with a sterile 2 in. borer (vertically from top to bottom) for bacteriological examination.

The holes made by the sample auger were filled with bran after sampling, and it was found that there was no waste or loss of quality in the surrounding silage when this method of sealing was adopted. After 3½ months the silos were emptied, their contents sampled, analysed and submitted to digestibility trials.

The materials added to the grass in the different silos were as follows:

- (1) Dried whey inoculated with lactobacilli.
- (2) A solution of dried whey<sup>1</sup> inoculated with lactobacilli.
- (3) A solution of dried whey<sup>1</sup> alone.
- (4) Fresh whey<sup>1</sup> inoculated with lactobacilli.
- (5) Mineral acid calculated to lower the pH to a value between 3.0 and 4.0.
- (6) A solution of molasses inoculated with lactobacilli.

The whey in treatments Nos. 1, 2 and 3 was added at a rate sufficient to supply 1 lb. of lactose per 100 lb. of green fodder. The fresh whey in treatment No. 4 was added at a heavier rate, 150 gallons being used per ton of fresh material; a good deal of this was lost in the drainage effluent during filling. In treatment No. 5 a mixture of mineral acids consisting principally of hydrochloric was used; this, however, did not bring about

<sup>1</sup> The use of whey was suggested by Captain Golding (6), to whom the authors are indebted for the supply of both the whey and the cultures.

the desired acidity and, though of interest, did not fulfil the conditions required of it, i.e. reduction of the pH to below 4.0. Molasses in treatment No. 6 was added at the rate of 2 lb. per 100 lb. of fresh grass. The culture used in all cases was the same—a mixed culture of *L. acidophilus*, *L. bulgaricus* and *L. casei*.

Silage No. 6 was made in a silo which had no provision for drainage. In all other cases conditions of free drainage obtained.

#### BACTERIOLOGICAL EXAMINATION

Each sample boring was first disintegrated and thoroughly mixed and then extracts and dilutions were prepared by methods previously given. Inoculations were made from these into the following media:

- (1) Dextrose-bean-extract-agar plates incubated aerobically at 30° C.
- (2) Yeast-extract-dextrose broth incubated anaerobically at 37° C.
- (3) Yeast-extract-whey-agar shake cultures at pH 4.2, incubated at 37° C. (Marmite was used as a convenient form of yeast extract.)

Medium (1) allowed the growth of a varied aerobic flora, though later experiments showed that it frequently does not give such a high count as yeast-extract-dextrose agar. Medium (2) allowed the growth mainly of lactobacilli and to a certain extent in the lower dilutions of obligate anaerobes and yeasts. In medium (3) lactobacilli were found almost exclusively though the counts represented only a fraction (the more acid-resisting portion) of the true numbers present in the silage. No special medium was used for obligate anaerobic spore-formers.

The numbers of bacteria per gram of silage in different media at intervals during the ripening is shown in Table 1.

The type of flora developing in the different silages was determined by making stained preparations of the growths in each medium and subculturing representative strains into either litmus milk or yeast-extract-litmus milk or on to dextrose-bean agar. In this way a differential count of the main groups of organisms present at each stage of the ripening silage was obtained and in the course of the whole investigation about 400 cultures were isolated for detailed study.

The changes in flora taking place in the different silages are summarized below.

#### *Treatment No. 1 (dried whey containing culture)*

Lactobacilli of various fermentation reactions, the growth of which was usually stimulated to a marked extent by the addition of yeast extract to the medium, were predominant in the early stages, attaining

Table I. *Total count of bacteria on various media. (Millions per gram of silage)*

Age of silage in days	Dried whey with culture implanted				Solution of dried whey plus culture				Solution of dried whey alone				Ordinary whey plus culture				Molasses plus culture				Acid process			
	Mar.				Mar.				Mar.				Mar.				Mar.				Mar.			
	Dex-trose bean agar pH 4.2	mite whey	2% dex-trose broth	2% dex-trose broth	Dex-trose bean agar pH 4.2	mite whey	2% dex-trose broth	2% dex-trose broth	Dex-trose bean agar pH 4.2	mite whey	2% dex-trose broth	2% dex-trose broth	Dex-trose bean agar pH 4.2	mite whey	2% dex-trose broth	2% dex-trose broth	Dex-trose bean agar pH 4.2	mite whey	2% dex-trose broth	2% dex-trose broth	Age of silage in days	Dex-trose bean agar	mite whey	2% dex-trose broth
5	145	70	100	242	75	1,000	1,000	1,000	760	135	1000	1000	269	34	1000	3170	500	100	—	—	—	—	—	—
8	28.4	8.3	10	376	81	100	100	100	315	47	100	100	129	8.2	100	42.1	13	1000	3	15.8	—	—	—	10
13	17.3	72	100	10.1	200	10,000	10,000	10,000	523	440	1000	1000	14	3	100	730	290	100	8	660	—	—	—	10,000
21	28.4	45	1000	564	320	1,000	1,000	1,000	94	88	1000	1000	—	32	100	39	240	1000	16	0.34	3.2	—	—	1
32	1.66	75	100	0.17	160	100	100	100	1.3	57	100	100	71,000	91	100	0.02	148	100	27	0.16	5	—	—	10
46	18.7	77	100	1.5	54	10	10	100	—	7.9	100	100	—	28	1	23.5	129	1	41	0.37	2.6	—	—	100
60	—	17	—	—	92	—	—	—	—	99	—	—	—	6.6	37	—	0.74	52	—	—	—	—	—	—

## 298     *Bacterial and Chemical Changes in Grass Silage*

a number of 100,000,000 per g. after 5 days. After 8 days, however, there was a considerable decrease in the count of these organisms and the flora consisted mainly of aerobic spore-formers of the *B. mesentericus* type. Lactobacilli then became increasingly numerous until, after 45 days, there were very few other types present. The proportion of lactobacilli able to coagulate plain litmus milk was low in the early stages but increased towards the end.

### *Treatment No. 2 (solution of dried whey containing culture)*

After 5 days the flora on dextrose-bean agar showed the presence of about 44,000,000 yeasts (per gram of silage) of differing fermenting powers and about 11,000,000 coliforms, with smaller numbers of aerobic spore-formers. Lactobacilli which actively fermented lactose and were able to grow well in milk were, however, predominant at this early stage and in later stages constituted almost the entire flora of the silage. More than 90 per cent of the lactobacilli, isolated after various intervals from this type of silage, were found to produce an acid clot in litmus milk without yeast extract.

### *Treatment No. 3 (solution of dried whey alone)*

The total number of bacteria present after 5 days was higher than in most of the later stages. For the first 21 days appreciable numbers of yeasts and micrococci were detected, though not in numbers approaching the lactobacilli. The latter were predominant throughout and nearly all the strains tested fermented lactose and grew well in milk. After 21 days the numbers of other micro-organisms were negligible.

### *Treatment No. 4 (fresh whey containing culture)*

The flora showed more fluctuation in this type of silage than in the others. After 5 days yeasts, coliforms and aerobic spore-formers were present in considerable numbers, though lactobacilli of the type giving an acid clot in litmus milk were predominant. After 8 days there was a decrease in numbers, mainly in the lactobacilli, and the latter were approximately equalled in numbers by yeasts and spore-formers. The flora on the dextrose-bean-agar plates declined rapidly until after 21 days there were less than 10,000 per g. On the thirty-second day these plates showed a sudden large increase in numbers of bacteria to 71,000,000,000 per g. These consisted almost entirely of a Gram-positive, yellow micrococcus, forming capsules on a dextrose medium, slowly liquefying gelatin, reducing nitrates to nitrites, and having very little effect on carbohydrates.

At subsequent stages the numbers of bacteria on the plates were again very low. There were between 100 and 1000 million lactobacilli per g. for the first 32 days, the numbers declining considerably later. Most species produced an acid clot in litmus milk.

*Treatment No. 5 (mineral acid)*

In the early stages the counts on all media were comparatively very low. After 8 days there was a very large increase in numbers comprising a mixed flora of yeasts, aerobic spore-formers and microaerophilic rods which produced an alkaline coagulation in litmus milk. No lactobacilli appeared until after 16 days, but henceforth they were predominant though present in comparatively small numbers.

*Treatment No. 6 (molasses containing culture)*

After 5 days there was a very high count on the dextrose-bean-agar plates, consisting mainly of non-sporing rods producing only slight acid in sugar broths and no change in litmus milk. Numbers on these plates decreased subsequently to a very small figure. Lactobacilli were present in large numbers throughout and in the later stages constituted practically the entire flora. Of thirty-six strains isolated at various stages during the ripening of the silage all but three quickly produced an acid clot in plain litmus milk.

CHEMICAL EXAMINATION

*Composition.* All six samples of silage were of a light brown-yellow colour, with a pleasant smell and faintly acid taste. They would all be classed as excellent, and were readily eaten by sheep and cattle. During the filling of the silos, samples representative of the fresh grass were taken, one for each silo. Again, when the silos were emptied, they were carefully sampled, and a bulk sample was made up for each silo. The fresh grass and silage samples were carefully subsampled for dry-matter determination, and the residue of the sample used for certain determinations which were carried out on the fresh material. To ensure proper sampling, the whole of each sample was put through a small laboratory chaff-cutter and then well mixed.

The following determinations were made on the fresh silage: The pH was determined on juice expressed in a simple tincture press, using a quinhydrone electrode. The total acidity, amino acids, and volatile bases and acids were determined in the fresh material by the Woodman modification<sup>(7)</sup> of the method of Foreman<sup>(8)</sup>. The difference between the

### 300 *Bacterial and Chemical Changes in Grass Silage*

total acidity and the sum of the amino-acid and volatile-acid values may be assumed to be due mainly to lactic acid in the case of silages to which no mineral acids have been added(9). The values are summarized in Table II.

Table II. *Total acidity, volatile constituents, lactic-acid and amino-acid contents of silages made with added whey or molasses with a bacterial culture. (Per 100 g. fresh silage)*

	Dried whey with culture implanted	Solution of dried whey plus culture	Solution of dried whey alone	Ordinary whey plus culture	Molasses plus culture	Added acid
pH	4.12	3.83	3.84	3.66	3.73	4.06
Total acidity c.c. N/10 acid	417.9	422.9	447.4	441.2	467.1	296.3
Amino acids as crude protein (g.)	1.01	1.00	0.99	0.96	1.02	0.79
Volatile bases as crude protein (g.)	0.25	0.25	0.19	0.13	0.17	0.20
Volatile bases as ammonia (g.)	0.048	0.048	0.037	0.026	0.033	0.039
Volatile acids as acetic acid (g.)	0.67	0.60	0.45	0.37	0.38	0.41
Residual acidity as lactic acid (g.)	1.72	1.88	2.33	2.43	2.58	1.23

The acidities of the samples with added whey, and with molasses, were satisfactory, the only pH value over 4.0 being that of the silage made with dried whey with implanted culture. It should be realized that as this material was added as a solid and not in solution, it would not be so well distributed throughout the material, and the stimulus given to the lactic fermentation would not permeate the whole mass as in the other cases. Despite this, the lactic-acid content was very high, and the silage was of really excellent quality. The figure for volatile acids was not high in any of the silages, including that made with added acid. It is evident that the major part of the breakdown products was in a useful form, mostly as amino acids, whilst the volatile bases were low. The sample made with added acid was similar in most respects to the other silages, the formation of amino acids being somewhat less than in the other types, but the volatile bases are no less. The residual acidity in the last-named silage has been calculated as lactic acid, though it would not all be present as such in the material.

The samples used for dry-matter determination were ground and analysed in the usual way. In addition, the volatile acids and bases remaining in the dried sample were estimated to give an accurate measure of the amounts lost during drying by comparing the values with those determined on the fresh silages. These experiments have been quoted already in a preliminary report(10), the figures used then being the values uncorrected for volatile material lost during drying. In Table III the average values for the composition of the fresh grass filled into all five silos is given, together with the digestibility coefficients, and is compared

Table III. *Composition and digestibility of fresh grass and silage.*  
(*Stated as percentages of the dry matter*)

	Fresh grass		Dried whey with culture implanted		Solution of dried whey plus culture		Solution of dried whey alone		Ordinary whey plus culture		Molasses plus culture	
	Compo- sition	Digesti- bility	Compo- sition	Digesti- bility	Compo- sition	Digesti- bility	Compo- sition	Digesti- bility	Compo- sition	Digesti- bility	Compo- sition	Digesti- bility
Ether extract	2.51	35.1	6.27	67.4	5.76	73.2	5.23	67.3	5.06	71.4	4.92	64.
Fibre	24.22	75.1	24.68	81.6	24.28	83.3	25.48	83.0	24.31	82.6	24.35	80.
Crude protein	10.66	66.3	13.50	69.2	13.96	72.8	13.04	69.6	12.57	69.1	12.56	71.
Ash	7.79	—	9.49	—	9.70	—	9.45	—	9.01	—	9.03	—
N-free extractives	54.82	78.9	46.06	79.0	46.30	80.6	46.80	79.1	49.05	80.5	49.14	80.
"True" protein	9.14	63.0	7.69	53.0	6.71	54.6	6.92	52.9	7.34	52.4	7.35	31.
Ratio "True" protein Crude protein	0.86	—	0.57	—	0.48	—	0.53	—	0.58	—	0.59	—
Starch equivalent	60.1	—	57.2	—	58.4	—	57.8	—	60.3	—	58.1	—
Starch equivalent (corrected)	—	—	60.4	—	62.8	—	61.3	—	63.3	—	62.8	—
Digestible crude protein	7.07	—	9.34	—	10.16	—	9.08	—	8.69	—	8.97	—
Digestible "true" protein	6.13	—	4.08	—	3.66	—	3.66	—	3.85	—	2.31	—
Digestible "true" protein (corrected)	—	—	7.56	—	8.24	—	7.46	—	7.04	—	7.30	—
Dry matter in fresh material	26.3	73.3	26.2	75.4	25.8	77.8	25.8	76.4	26.1	77.1	24.9	76.



## 302 *Bacterial and Chemical Changes in Grass Silage*

with the composition of the silages, the material made with added acid being omitted. The volatile acids lost on drying have been added to the ether-extract fraction, whilst the volatile bases are added to the crude protein.

The figures show that the ether extract rose in all silages, due in a great measure to the increase in organic acids, and that this fraction was more digestible in silage. The fibre was apparently slightly more digestible in the silage than the fresh grass, though the proportion was the same in both. The crude protein was higher in the silage than the original grass, and the digestibility of the protein was not affected by the silage process.

It is in the N-free extractives that the greatest differences are found, the ensilage process resulting in a loss of this fraction, though the digestibility did not suffer. The "true" protein was broken down, as is shown most clearly by the ratio of "true" to crude protein, but there was no great difference between the different silages.

The starch-equivalent values of the samples of silage, which were calculated by the method of Kellner<sup>(11)</sup>, showed no great differences from that of the fresh grass. The corrected figures for starch equivalent are based on the corrected value for digestible "true" protein, which were calculated on the assumption that the breakdown products on the "true" protein are of full feeding value<sup>(12, 13)</sup>. On this basis, the silages showed higher values than the fresh grass.

The digestible crude protein was higher in the silage samples, as was the case with the crude protein itself. The digestible "true" protein values were lower in the silages as a result of the breakdown of the nitrogenous constituents, but when corrected the fresh grass again showed a lower value. The correction of the "true" protein figures was made by applying to the digestible crude protein the ratio which the fresh grass exhibited for digestible crude and digestible "true" protein.

The feeding values of all the samples of silage was similar to that of the fresh grass.

### LOSSES IN NUTRITIVE VALUES

Though the similarity between the fresh grass and the silages was so marked, it does not follow that there were no losses of nutritive value during ensilage; a common fallacy, since it is essential to have the weights of material filled into the silo and taken out, as well as the analysis.

The material was carefully weighed, and from these fresh weights and the known dry-matter content and analysis of the materials it is possible to calculate the losses. It is necessary, however, in estimating the weight of dry matter removed from each silo to make a correction

for the volatile material lost in drying the samples for analysis. The method of doing this has already been described. To give the correct picture, it is necessary to take into account the dry matter, nitrogen, ash, ether extract and N-free extractives added in the whey or molasses, and the culture. The position may be summarized as follows:

Table IV. *Balance-sheet for dry matter in different silages*

Type of silage	Dried whey with culture implanted	Solution of dried whey plus culture	Solution of dried whey	Ordinary whey plus culture	Molasses plus culture	Added acid
Dry matter in (lb.)	465.7	471.2	476.7	538.8	446.6	622.0
Dry matter in whey molasses (lb.)	24.0	28.8	26.0	44.4*	27.8	—
Total in (lb.)	489.7	500.0	502.7	583.2	474.4	622.0
Dry matter out (lb.)	376.1	397.7	420.1	482.9	434.7	471.0
Correction for volatiles (	10.0 (2.66)†	9.5 (2.37)†	6.9 (1.63)†	6.7 (1.39)†	5.3 (1.21)†	5.0 (1.06)†
Total out (lb.)	386.1	407.2	427.0	489.6	440.0	476.0
Loss (lb.)	103.6	92.8	75.7	93.6	34.4	146.0
Loss (%)	21.2	18.6	15.1	16.0	7.3	23.5

\* The whey ran out of the silo very rapidly, owing to the large volume used. It was assumed that 50 per cent was lost. If all were retained, the loss of dry matter would be 25.4 per cent.

† The figures in brackets show the percentage losses of volatile matter from the dry matter during the drying of the sample, and on these the correction for volatile material is based.

With the exception of the silage made with added molasses, the losses of dry matter were similar with all treatments. The molassed silage was made in a silo which had no drainage, and the lower losses in the material are no doubt due largely to this fact. The dry-matter losses are of the same order as those obtained in large silos containing up to 30 tons of silage (14), and somewhat higher than is often thought. It should be remembered that they include any side or top waste, although there was very little of this in any of the silos. It would be possible in most cases to prevent free drainage from the silos, in which case the losses would be lower and correspond more nearly with those for molasses silage. It is, however, usual to allow relatively free drainage in the case of additions of mineral acid and also where fresh whey is used.

It is possible to calculate the losses of the different ingredients from a knowledge of the composition of the fresh grass and of the different silages, together with the data for the weights of dry matter ensiled and those recovered. The whey was analysed, as was also the molasses, so that a correct balance-sheet could be drawn up for each type of silage. The silage made with added acid was not tested for digestibility, and is not included in the discussion of individual losses of constituents.

The losses in the molassed silage were lower than the whey silage, but the reason for this has already been noted; the silo did not have free drainage. The losses of starch equivalent were of the same order in all

### 304 *Bacterial and Chemical Changes in Grass Silage*

four whey silages, and were but little higher than those of dry matter, as was to be expected from the high starch-equivalent value of the silages. The main loss has fallen on the N-free extractives, whilst the ether extract showed a gain due to the inclusion of organic acids, products of fermentation, in this fraction. The figures for loss of protein are anomalous in some cases. It must be realized that the total amount of crude protein in the silo was only some 50 lb. A further difficulty exists in the fact that the figure for crude protein is obtained from the total nitrogen content by the application of the usual factor, and the resultant figure has again to be corrected for the loss of volatile bases during the drying of the sample—another source of error. The most that can be said of the protein loss figures is that they demonstrate that no appreciable loss of protein has occurred.

Table V. *Losses involved in the making of silage with added whey and molasses, with and without bacterial cultures, as percentages of the material in the fresh crop. (Gains as a positive sign)*

	Dried whey with culture implanted	Solution of dried whey plus culture	Solution of dried whey	Ordinary whey plus culture	Molasses plus culture
Ether extract	+ 86.2	+ 79.4	+ 65.2	+ 58.0	+ 120.4
Fibre	- 11.3	- 11.9	- 9.3	- 7.5	- 4.5
Crude protein	- 6.8	+ 3.5	+ 10.7	- 6.4	+ 14.5
Ash	- 9.0	- 0.5	+ 5.5	- 2.0	+ 7.9
N-free extractives	- 34.9	- 32.7	- 28.8	- 26.8	- 19.1
Digestible crude protein	- 4.8	+ 10.7	+ 13.1	- 6.0	+ 22.3
Digestible "true" protein	- 50.0	- 52.1	- 46.4	- 50.1	- 60.7
Digestible "true" protein (corrected)	- 7.0	+ 8.0	+ 9.7	- 8.5	+ 24.9
Starch equivalent	- 26.7	- 23.1	- 20.3	- 19.0	- 10.6
Starch equivalent (corrected)	- 22.6	- 17.3	- 15.4	- 15.0	- 3.4

This difficulty does not arise in the determination of dry matter N-free extractives or starch-equivalent losses, and the accuracy of these values will be much greater, being subject only to sampling error.

The silage made with fresh whey (7.1 per cent dry matter) called for an addition of 125 gallons for the 18 cwt. of fresh grass ensiled. This is too large an amount and, indeed, the drain was running fast almost from the commencement of filling, and a very large proportion of the whey must have been lost almost at once. In calculating the loss of dry matter, it was assumed that 50 per cent of the whey was retained and was effective. It is therefore possible that the amount used might be reduced. Even this, some 60–70 gallons per ton, is a large addition, particularly if the crop be at all wet. The excessive amount of water to be added might prevent

any heating and result in undesirable fermentations. This silage was the least satisfactory, though still of excellent quality.

Gerlach & Gunther (15) have used whey as an addition to serradella made into silage in a small container holding about 2000 lb. of chaffed material, to which whey was added at the rate of 4 lb. per 100 lb. The resultant silage was free from butyric acid, contained 0.60–0.68 per cent of lactic acid, 0.34–0.37 per cent of acetic acid, and 0.043–0.053 per cent of ammonia in the fresh material. The acids are somewhat lower than has been found in the present series of experiments, but the ammonia is of a similar order.

Kirsch *et al.* (16) have also carried out tests using small silos which held 4 cwt. of fresh clover, to which about 7 lb. of whey were added per 100 lb. of fresh clover. Fresh whey was tested, as was also whey from which the protein was removed by coagulation by heat. Heating would destroy the bacteria, and in one treatment protein-free whey was used alone, whilst in another it was inoculated with lactic organisms. The pH of the resultant silages was about 4.9, and the lactic acid content was low in all (0.12–0.30 per cent of the fresh silage), being highest where a culture was added to the protein-free whey. The acetic acid ranged from 0.77 to 1.07 per cent, which is high, and butyric acid was present (0.36–0.83 per cent), being lowest in the silage made with added protein-free whey.

These results are not favourable, and the authors in fact suggest that whey should not be used for making silage.

Gerlach & Gunther did not determine the digestibility of the material. They report gains of 126 per cent of ether extract, 5.46 per cent of crude protein, 17.8 per cent of digestible crude protein, and losses 4.92 per cent of N-free extractives, 2.79 per cent of fibre, 6.55 per cent of ash, and 32.9 per cent of “true” protein. The figures for N-free extractives are considerably lower than our own figures; the “true” protein shows a loss of a slightly lower order, and the crude protein shows a gain, from which it may be implied that there was no great loss of this constituent, nor indeed of any except the “true” protein.

Kirsch and his co-workers measured the digestibility of the silages, and quote the following losses for starch equivalent and digestible crude protein.

	Starch equivalent	Digestible crude protein
Silage with added whey	– 21.9	– 21.0
Silage with protein-free whey + culture	– 19.8	– 19.3
Silage with protein-free whey	– 22.6	– 23.0

The starch-equivalent losses are similar to the figures obtained in the present series of trials, but the digestible crude-protein losses are considerably higher in the German work.

## CONCLUSIONS

These results, viewed in the light of data obtained from the previous experiments on uninoculated silage<sup>(1)</sup>, point the way to some important conclusions which may be tabulated as follows.

The addition of whey and molasses to material for ensilage results in a material of high lactic acid content and production of a silage of excellent quality and digestibility and of suitable acidity. The losses of nutritive value were of the same order in all the silages where free drainage existed. The main loss occurred in the N-free extractives. The protein was retained in a large measure. Fresh whey is not so satisfactory as concentrated or dried whey diluted to a suitable concentration, since the volume of the former to be added is often excessive, and with a young succulent crop may prove dangerous to satisfactory fermentation.

The numbers of micro-organisms in the silages made with various additions were considerably higher (particularly in the early stages) than those observed previously in normal grass silage<sup>(1)</sup>. This may be due partly to the fact that the latter was made from a particularly dry grass whereas the former were made from grass which was initially much moister and the different treatments to which it was subjected involved the addition of an appreciable extra quantity of moisture. It is also no doubt due to the influence of the extra supply of carbohydrate contained in the added materials, since treatment No. 1, which consisted in the addition of dried whey without moisture, showed higher counts, though not so high as in the silages to which moisture was added.

The pH finally attained by each treated silage (average about 3.8) was considerably lower than that found previously for untreated silage (average for test-tube silage 4.2, for field silos 5.2).

The essential lactic-acid flora was well maintained in the treated silages but the species of lactobacilli predominating were different in one important respect from those found throughout in the untreated silages reported earlier<sup>(1)</sup>—i.e. they were in all cases mainly active fermenters of lactose and grew comparatively rapidly in milk to produce an acid clot without the stimulus of yeast extract—a fact which sharply distinguishes them from the *Streptobacterium plantarum* type indigenous to grass and forming the chief flora in normal grass silage. Moreover, throughout the period of ripening of the silage the proportion of the total number of lactobacilli which were acid-resistant was much higher in the treated silage than in normal, untreated silage<sup>(1)</sup>. That this difference was not by any means entirely due to the fact that some of the silages were inoculated

with this species of lactobacillus may be deduced from the fact that treatment No. 3 (addition of the solution of dried whey containing no inoculum) resulted in the appearance of the same type of lactobacillus as in other treated silages.

We may combine the above observations to conclude that additions of carbohydrates in an available form combined in some cases with an inoculum of lactobacilli, resulted in the growth of larger numbers of lactobacilli than occurs in untreated silage, and that these were of a different character. This in turn resulted in a greater development of lactic acid and a reduction of the  $pH$  to a value lower than that normally observed.

Streptococci have not been observed, in any of the silages investigated, to attain more than negligible numbers, and this applies even to whey silage where lactic acid streptococci must have been present in large numbers in the material added. It therefore appears that streptococci play no part in the ripening of grass silage and that no useful purpose would be served by using them as an inoculum. Such a conclusion is to be expected on general grounds since the changes taking place in silage normally result in the rapid reduction of the  $pH$  to a value inhibitory to the growth of streptococci.

Of the treated silages examined that made with the addition of whey in large quantities (treatment No. 4) showed the greatest fluctuation in flora, and in at least one stage, lactic-acid bacteria were outnumbered by other types. This indicates the possible danger of adding too large a quantity of fluid, containing, in addition to a large amount of available carbohydrate, a very varied flora which can utilize it.

We are justified in drawing from these observations the tentative conclusion that the addition of soluble carbohydrate to grass silage at the time of making may be beneficial by encouraging the formation of lactic acid which reduces the  $pH$  to a low value. Care must be taken at the same time, however, to avoid the simultaneous inclusion of a large mixed flora which may develop subsequently in a way which is detrimental to the predominance of the lactic acid fermentation. Experiments so far have indicated that molasses is a suitable source of carbohydrate. The subsequent development of lactobacilli may be further ensured by inoculating the molasses with a suitable culture and for this purpose it is probable that a mixed culture of lactobacilli of the *Streptobacterium plantarum* type, found by experiment to ferment the carbohydrates in molasses, would be the most beneficial.

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# A NOTE ON THE EFFECT OF DIFFERENT CEREALS IN THE FATTENING RATION ON THE COMPOSITION OF THE BODY FAT OF THE FOWL

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THE object of fattening poultry is to obtain increased carcass weight, and to improve the quality of the product by inducing the deposition of fat, particularly intramuscular fat, which makes the flesh more moist and tender. It is evident that the nature of the fat laid down will exert an influence on the flavour and succulence of the flesh and therefore on its market value. It has been shown<sup>(1)</sup> that a close relationship exists between the fats contained in a ration and the fats deposited in the body of the fowl, the ingestion of a fat of low iodine value producing a harder fat than normal, while fats of high iodine value produce body fat which is markedly softer than normal.

From the practical standpoint it is of interest to ascertain whether the type of cereal used in poultry-fattening rations has any significant effect on the consistency of the body fat deposited during the fattening period, since in this country it is popularly supposed that maize, owing to the unsaturated nature of the oil which it contains, produces an undesirably soft fat in the finished carcass. This opinion is based on the fact that maize feeding exerts a softening effect on the body fat in pigs. With the object of obtaining information on this point, which is of considerable importance in poultry production, fattening experiments were carried out with rations containing different cereals which are commonly used in poultry fattening mixtures, and the composition of the body fat produced on these rations was investigated.

## EXPERIMENTAL

The cereals tested were maize, oats and barley. The amount of oil which these contain and the approximate chemical composition of the oils are shown in Table I.

Each fattening ration contained 12 parts of dried skim milk and 88 parts of the cereal to be tested.

The birds used were Light Sussex cockerels reared on a normal stock



### 310 *Effect of Fattening Ration on Body Fat of Fowl*

ration with access to free range. Two sets of experiments were run, each set consisting of three groups each containing six cockerels. No. 1 was carried out in November with mature birds; No. 2 was carried out in July with considerably younger birds.

The cockerels were trough fed for 12 days, the rations being mixed with water to a suitable consistency. At the end of this period the birds were starved for 24 hours, weighed and killed, and samples of the body fat were removed. The samples from the individual birds in each group were pooled for analysis.

#### *Methods of analysis*

*Cereal oils.* In the case of Sussex ground oats, the oil was extracted with petrol ether according to the method of Amberger & Hill (2), while the oil of barley and of maize was obtained by prolonged extraction with anhydrous ether in a Bolton and Revis extractor. It proved difficult to obtain clear extracts of the oils, owing to the presence of very finely divided material, possibly carbohydrate in nature, which caused slight turbidity. To remove this, the ether was evaporated off, the residue taken up in acetone and allowed to stand for a few hours before filtering. This process had to be repeated several times in the case of oat oil.

*Body fat.* The fat was extracted by boiling with acetone, which was then distilled off, the last traces being removed *in vacuo* at 100° C. The solid and liquid acids were separated according to Twitchell's method (3), viz. by crystallization of the lead salts from 95 per cent alcohol using the procedure of Hilditch & Priestman (4). The iodine value of the solid acids was estimated and the percentage of solid acids corrected for any oleic acid present. Oleic and linoleic acids were calculated from the iodine value of the mixed acids after allowance had been made for the solid acids present.

Table I. *Chemical composition of cereal oils*

		Oats	Barley	Maize
Oil	i.v.	103.4	112.9	112.9
	Sap. no.	190.1	181.1	188.0
Mixed acids	i.v.	108.0	121.9	118.0
Solid acids	%	12.0	15.6	12.1
	Mol. wt.	260.0	265.4	257.6
Liquid acids	i.v.	122.1	144.9	135.4
	Mol. wt.	285.2	286.3	286.2

#### *Percentage composition of mixed fatty acids*

Solid acids	12.0	15.6	12.1
Oleic acid	56.4	33.9	45.2
Linoleic acid	31.6	50.5	42.7
Percentage of oil in grain	4.5	2.5	4.1

The maize oil obtained was a deep orange-yellow colour, the barley a brownish yellow and the oat oil a greenish yellow. All three were slightly viscous at room temperature.

Table I shows that all three oils were highly unsaturated. The mixed fatty acids of maize and of barley have approximately similar iodine values, but the barley acids contain the higher percentage of linoleic acid. The linoleic acid content in both cases is appreciably greater than in oats. The thiocyanogen value was determined on the liquid acids according to the method of Kaufmann & Keller(5) but no linolenic acid could be detected in any of the samples. Täufel & Rusch(6), however, record that the mixed fatty acids of barley oil contain 0.5 per cent of linolenic acid.

Table II. *Exp. 1. Live-weight gains during fattening (mature birds)*

Group 1. Ration: 88 parts Sussex ground oats, 12 parts dried skim milk.

Food consumption 39 lb.

No. of bird	Initial wt. g.	Final wt. g.	Increase in wt. g.
262	2808	2890	82
55	3170	3516	346
291	2890	3192	302
472	2902	3008	206
433	3198	3304	106
444	2710	2890	180

Average 203.6

Group 2. Ration: 88 parts maize meal, 12 parts dried skim milk.

Food consumption 33 lb.

No. of bird	Initial wt. g.	Final wt. g.	Increase in wt. g.
351	2940	2984	44
220	3060	3190	130
454	2786	2800	14
371	3238	3320	82
255	2936	2984	48
311	3006	3005	— 1 g. loss

Average 52.8

Group 3. Ration: 88 parts barley meal, 12 parts dried skim milk.

Food consumption 39 lb.

No. of bird	Initial wt. g.	Final wt. g.	Increase in wt. g.
464	2832	3018	186
425	2920	3158	238
204	2700	2830	130
113	2640	2844	204
122	2820	2780	— 40 g. loss
208	2940	3194	254

Average 162

Since the actual oil content of the grain fed as well as the composition of the oil determines the consistency of the body fat produced therefrom, it

## 312 *Effect of Fattening Ration on Body Fat of Fowl*

should be noted that though barley oil contains the highest percentage of linoleic acid the oil content of the grain is considerably less than that of oats or maize.

Table III. *Exp. 2. Live-weight gains during fattening (immature birds)*

Group 1. Ration: 88 parts Sussex ground oats, 12 parts dried skim milk.  
Food consumption 31.5 lb.

No. of bird	Initial wt. g.	Final wt. g.	Increase in wt. g.
64	2030	2604	574
46	1764	1708	— 56 g. loss
86	1568	1708	140
122	1932	2492	560
147	1624	2044	420
148	1932	2240	308
Average 324.3			

Group 2. Ration: 88 parts maize meal, 12 parts dried skim milk.  
Food consumption 33 lb.

No. of bird	Initial wt. g.	Final wt. g.	Increase in wt. g.
58	1708	1736	28
93	1792	2002	20
113	1932	2198	266
114	1932	2338	408
138	1960	2254	294
145	1764	2044	280
Average 216			

Group 3. Ration: 88 parts barley meal, 12 parts dried skim milk.  
Food consumption 39 lb.

No. of bird	Initial wt. g.	Final wt. g.	Increase in wt. g.
92	1988	2128	140
94	1820	2016	196
96	1764	2156	392
101	1736	2044	308
125	2016	2408	392
154	1624	2100	420
Average 308			

## DISCUSSION

Tables II and III show that the best live-weight gains during the 12-day fattening period were obtained with the younger birds. Though the numbers used are too small to permit of definite conclusions being drawn, the results indicate that of the three cereals used, Sussex ground oats produced the best increase in weight and maize the poorest. This finding is in accordance with the results of the table-poultry experiments at Wye(7). In the present experiment the birds appeared to find maize the least palatable of the three cereals.

Table IV. *Exp. 1. Composition of body fat (mature birds)*

		Maize group	Oats group	Barley group
Fat	I.v.	72.6	77.2	62.0
	Mol. wt.	284.3	284.9	286.4
Mixed acids	I.v.	75.7	79.9	65.5
Solid acids	%	34.4	32.2	37.9
	Mol. wt.	265.3	267.7	263.6
Liquid acids	I.v.	113.8	115.2	103.1
	Mol. wt.	280.4	283.5	285.2

*Percentage composition of mixed fatty acids*

Solid acids	34.4	32.2	37.9
Oleic acid	47.2	47.1	51.5
Linoleic acid	18.4	20.7	10.6

Table V. *Exp. 2. Composition of body fat (immature birds)*

		Maize group	Oats group	Barley group
Fat	I.v.	64.0	61.5	59.8
	Mol. wt.	284.2	280.5	282.6
Mixed acids	I.v.	67.3	63.4	61.6
Solid acids	%	34.7	36.4	38.6
	Mol. wt.	267.6	266.6	260.6
Liquid acids	I.v.	102.6	100.9	100.2
	Mol. wt.	281.9	281.8	283.1

*Percentage composition of mixed fatty acids*

Solid acids	34.7	36.4	38.6
Oleic acid	55.9	56.9	54.5
Linoleic acid	9.4	6.7	6.9

The analysis of the fat of the mature birds, as shown in Table IV, shows that Sussex ground oats has produced the softest fat, i.e. the fat having the highest iodine value, the lowest percentage of solid acids and the highest percentage of linoleic acid. The body fat of the maize-fed birds is, however, very similar in composition. The body fat produced on the barley ration shows a significantly lower iodine value, a slight increase in the solid acid content, and a decrease in the percentage of linoleic acid present. While the fatty acids of the barley oil contain more linoleic acid than those of the other two cereals, and might on this account be expected to produce a more unsaturated fat, the amount of oil present in the grain is less, so that the body fat in the barley group was formed more exclusively from carbohydrate than in the other two groups. The consistency of the fat is practically normal in the maize and oat groups as compared with the body fat of birds reared on a ration of mixed cereals plus protein, where the iodine value of the fat is about 77–80 and the solid-acid content

### 314 *Effect of Fattening Ration on Body Fat of Fowl*

about 33 per cent<sup>(8)</sup>. The body fat of the barley group is more saturated than the normal.

In Exp. 2, where the live-weight increases were greater than in the mature birds, the body fat in all three groups is very similar, though the maize fat is slightly softer than the other two (Table V). It is worthy of note that in the younger birds all three cereals have produced body fats of significantly lower iodine value than the normal, and that the percentage of linoleic acid in the fats, especially in the maize and oat groups, has been considerably reduced as compared with the corresponding body fats in Exp. 1. This is presumably due to the fact that, since the weight increases during the period were greater, i.e. the fattening was more rapid, a greater proportion of the fat had to be formed from the carbohydrate fraction of the food. In pigs it has also been found that where the rate of fattening is rapid the fat tends to be more saturated, i.e. harder, than when fattening is prolonged<sup>(9)</sup>. The fat in the barley group is of very similar composition in both Exps. 1 and 2 since, owing to the small percentage of oil present in this cereal, the fat deposited was probably formed in both cases almost entirely from carbohydrate.

Judging from these results, therefore, it appears that the popular objection in this country to the use of maize in poultry-fattening rations is unjustified, since over a 12-day fattening period the maize ration did not lead to the production of an undesirably soft fat. Indeed, in Exp. 2 the consistency of the fat was harder than in normal unfattened birds, and in both experiments it was very similar to that produced on the Sussex ground oats ration.

The reason for the difference in the results obtained with fowls and in those obtained with pigs lies in the fact that in the former case the fattening period is short, seldom lasting more than 12–18 days, while in pigs the fattening process is more gradual and may extend over 2–3 months, during which time a considerable proportion of the body fat will be formed from the fat fraction of the food and in consequence will have a softer consistency than fat synthesized more exclusively from carbohydrate.

#### SUMMARY

Over a 12-day fattening period the use of maize in the poultry-fattening ration has no detrimental effect on the consistency of the body fat.

When fattening is rapid and live-weight gains are satisfactory, the type of fat deposited in the bird is harder than that laid down on a normal ration containing mixed cereals and a protein supplement.

## ACKNOWLEDGEMENTS

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## A STUDY OF THE FASTING METABOLISM OF VARIOUS BREEDS OF HOG

### III. METABOLISM AND SURFACE AREA MEASUREMENTS

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(With Four Text-figures)

IN this work the metabolism of twenty-five pigs in all has been measured from time to time at ages ranging from about 50 days to upwards of 2 years—considerably beyond the point therefore at which they would normally be marketed. Preliminary papers on this investigation have already appeared (1, 2), dealing with the general growth of the animals and with the variation of body temperature found.

This paper deals principally with the data accumulated on the metabolism, but a word must first be said as to the surface area of the pigs. For this there are two convenient methods available for animals which cannot be slaughtered: (a) the application of a formula, and (b) the photographic method worked out by the present writer (3).

The labour involved in the latter method is too great for general use in all the experiments, so the true area was determined by this method three times during the life of each of the pigs from pig R onwards. Pigs coming earlier in the series were observed a less number of times or not at all, as the method was only devised some time after the beginning of the investigation. (For details of the pigs by letter see paper I of this series.) For the areas at other times use was made of a composite formula:

$$S = 0.0093 W^{0.4} L^{0.6} + 0.0025 W^{0.3} L,$$

where  $S$  is the surface area in sq. metres,  $W$  = the weight in kg. and  $L$  the length in cm. This formula combines, with approximately equal weight, the ideas of Hogan & Skouby (4) and of Cowgill & Drabkin (5), with due allowance for deficiency in the former and the use of a different constant and length measurement in the latter as indicated by the present writer (3). The areas determined by this formula were corrected by



means of the values obtained by the photographic method, intermediate values being obtained by linear interpolation.

When the metabolism measured in the calorimeters in the usual manner (see previous publications (6, 7)) were computed per square metre in accordance with these surfaces, *no correspondence between surface and fasting katabolism could be found*. There seems to be no point in reproducing these diagrams—whatever the basis adopted for temperature correction there was no regularity about the final results at any age. On the other hand when the metabolisms were computed per  $9 \times (\text{weight})^{\frac{2}{3}}$ , some quite interesting points came out. It seems clear, however, that the relation is not directly one between the surface and the metabolism, but between a fractional power of the weight and the metabolism.

This represents a change of view on the writer's part, and one adopted not without considerable misgiving, since it would appear certain, on purely physical grounds, that the surface, *in some form*, must enter into the matter where the complete picture of the process of heat elimination in a warm-blooded animal can be drawn.<sup>1</sup> The view that surface area is not of primary importance in metabolism work has been advocated for many years by Benedict (10), and Kleiber (11) has shown that metabolism should preferably be related to a fractional power of the weight. He suggested that a value for this power should be adopted internationally and that some value between  $\frac{2}{3}$  and  $\frac{3}{4}$  should be chosen. Our results render it clear why the principal opponents of the "surface-law" tend to be found amongst those who use a height-weight or length-weight formula for surface, since these give a *closer* approximation to the true surface than formulae depending on weight alone, although by no means a close one, as has been shown by the writer (3). Kestner (12) has recently drawn attention to the relation existing between the development of the brain, liver, kidney and alimentary tract and metabolism. The connexion between brain weight and metabolism, and brain weight and body weight, is extraordinarily illuminating in this regard.

In Fig. 1 the fasting katabolism of each of the pigs is computed per  $9W^{\frac{2}{3}}$ , that is, in the same manner in which we were previously accustomed to compute metabolism per unit surface area, only that now the above expression is not considered to be the area. In order to bring all the results on to the one diagram, the abscissa has been divided according

<sup>1</sup> A. C. Burton (8) has made an interesting study of the physical implications of heat flow in the animal body, taking the blood flow into account, and is able to account satisfactorily for the fall of metabolism with advancing age; but it would seem inevitable that his equations should be revised to some extent if the results obtained by E. Borra (9) should be confirmed.

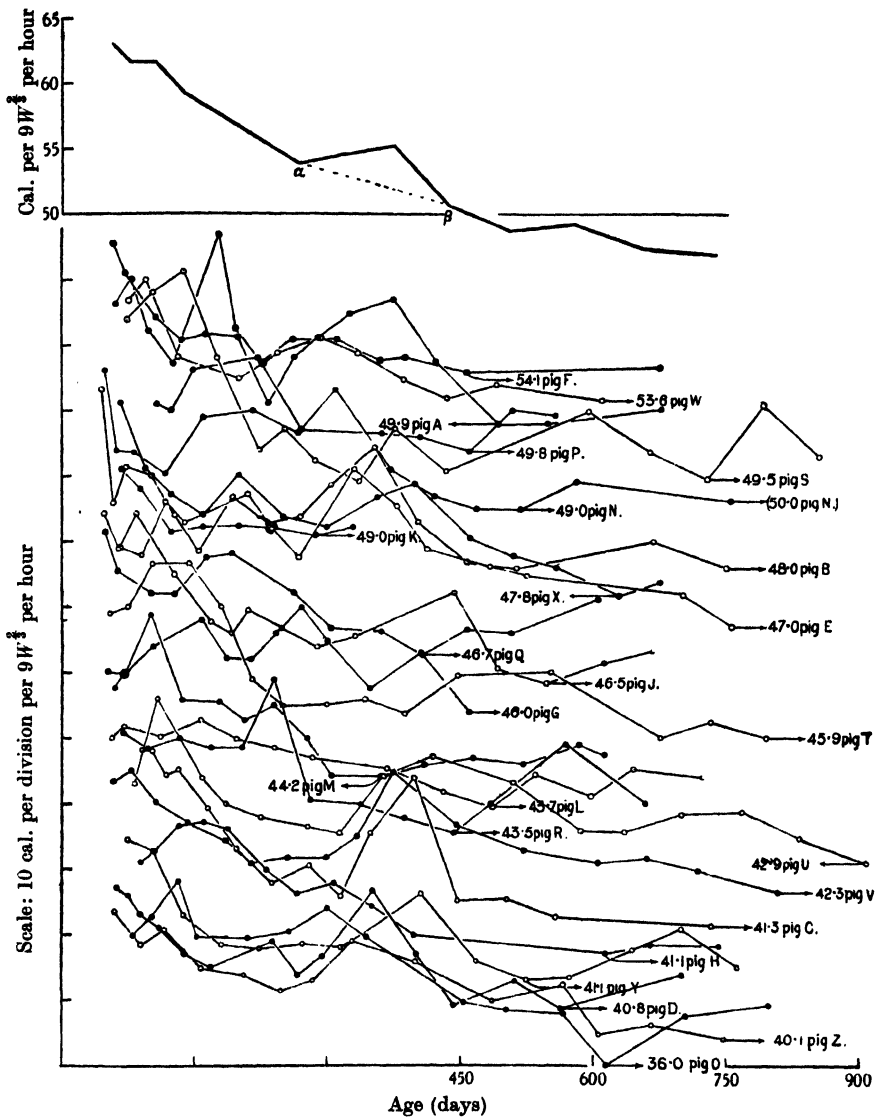


Fig. 1. Fasting metabolisms of all pigs, at ages 50—900 days, computed per 9 (weight)<sup>3</sup> per hour. Corrected for temperature differences by Capstick & Wood's curve. Top double line curve shows average of all pigs taken in age groups.

to age, while the ordinate is merely divided into calories without any definite zero. The values to be ascribed to the points on the curves are read on the ordinate scale from the lowest recorded observation for that particular pig. This is indicated by arrows on the chart and has its actual value appended.

A short examination of these curves shows that, apart from the maximum in early youth previously described, there is, in the conditions in which these pigs were kept, a tendency to a rise in the metabolism to a second maximum at an age of from 250 to 400 days. This has not been noted previously so far as I am aware, and it does not appear in all the pigs. On referring to previous publications of my own, I find that a similar phenomenon was actually observed before. As long ago as 1923, in preparing a curve of fasting katabolism per unit surface for a Large White pig, it was found necessary to reject one observation for the time being, as it failed to accord with the curve then thought to represent the course of variation of this quantity. Details of this observation were, of course, given in the published account<sup>(6)</sup>, and it now appears that this case was not exceptional and should be incorporated in the curve. A similar point was noted in one pig in a subsequent paper<sup>(7)</sup>.

The curve at the top of Fig. 1 shows the average value of fasting katabolism per 9 W<sup>2</sup> for all the pigs, and it will be noticed that in this case also the rise after the first fall is quite definite. It is shown still more clearly in Fig. 2, where the time along the abscissa, instead of being reckoned from the beginning of the experiment, is computed from the time of attainment of the *first* peak value.

With our instruments, which are not fitted with arrangements for gas-exchange observations, it has not been feasible to investigate this further. It may be said, however, that the instruments were frequently calibrated and there is no reason to doubt the existence of this second maximum other than its unexpectedness. Moreover, apart from the confirmation it receives from our own previously published work, it will be shown later in this paper that the *average* results in weight increase of eleven of the pigs, which were quite comparable with one another and in which conditions were uniform, agree remarkably well with the increase *computed* from the nett energy theory of Armsby, but only so long as these second maxima are taken as observed; if the curves are smoothed out and these high values ascribed to errors no agreement is found.

In seeking for some explanation of this phenomenon it seemed possible that the rise might correspond to the establishment of a higher plane of nutrition immediately following the periodical increases in the ration

fed. Reference to our laboratory notes showed, however, that this could not be so, since in approximately half the cases where the rise occurred the food supply had been augmented immediately before the rise began, and in the other half it had been constant.

More light was shed on the matter by a closer examination of the cases in which a second rise in metabolism took place and of those in which it did not or was at least doubtful. A comparison of these data with the date of birth of the pig showed very clearly that pigs born during the months May to September and early October, inclusive, show the second peak of metabolism during the following summer, while those born during the rest of the year either do not show it at all or do so in a very doubtful manner. A comparison of the data in the following table with the curves of Fig. 1 will render this sufficiently clear.

[In the case of pigs indicated by black letters there is no certainty about the month or even the real existence of the second peak.]

	Month of beginning of second peak of metabolism	Month of birth
Jan.	<b>M</b>	<b>M Q</b>
Feb.		<b>H R</b>
Mar.	<b>N S</b>	<b>S</b>
Apr.	<b>F L</b>	
May	<b>A U V W Y</b>	<b>T U</b>
June	<b>B D O Q R*</b>	
July	<b>C G J T Z</b>	<b>C</b>
Aug.	<b>E X</b>	<b>A D J V W X Y</b>
Sept.		<b>B E O</b>
Oct.		<b>F<sup>1</sup> N<sup>1</sup> Z<sup>2</sup></b>
Nov.		<b>G L</b>
Dec.	<b>N</b>	

<sup>1</sup> Oct. 19

<sup>2</sup> Oct. 10

The black letters in the second column refer to those pigs which either did not show this second peak or in which its existence was doubtful. Pig R, marked with an asterisk, was exceptional. In this pig both peaks occurred but were both very much later than usual in the life of the animal.

The pigs born from May to early October (col. 3) are all in ordinary type in the second column and reference may be made to Fig. 1.

A statistical investigation of the data of Fig. 1 in the neighbourhood of the rise in the top composite curve shows that while the actual rise above the horizontal at this point is not significant, that above the line  $\alpha\beta$ , taken as representing the apparent trend, is so. If, however, the pigs be divided into two groups, one, of thirteen pigs, born in the months May to September inclusive, and the other, of eleven pigs, born October to April inclusive, there is a fall in average fasting katabolism per 9 W<sup>†</sup> from 265 to 350 days in the latter group which does not differ significantly

## 322 *Fasting Metabolism of Various Breeds of Hog*

from the trend. On the other hand, with the thirteen summer and autumn pigs there is an actual rise above the *horizontal* whose significance is represented by  $t=3.31$ , where a 1 per cent significance requires  $t=3.055$ .

The most probable explanation of this second rise of metabolism in the summer following birth in pigs born in summer or autumn is possibly to be sought in an effect of light on pigs analogous to that found in many

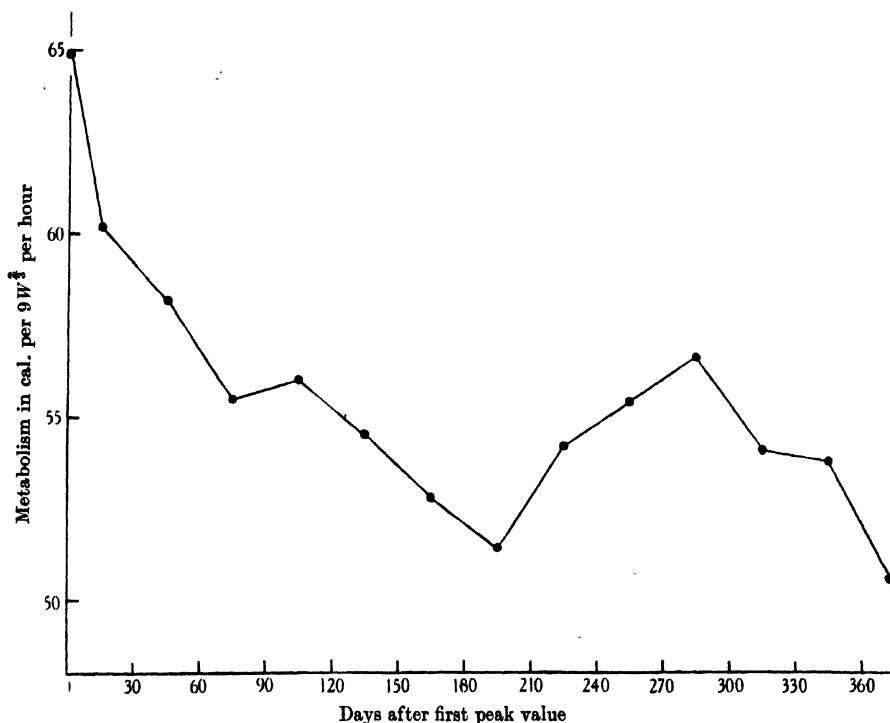


Fig. 2. Average fasting katabolism of all pigs plotted against time reckoned in days subsequent to the occurrence of the first peak value.

other animals and birds, due to a stimulation of the thyroid via the pituitary. That light does stimulate the pituitary has been rendered probable by Hill & Parkes<sup>(13)</sup> and by Marshall & Bowden<sup>(14)</sup>, while the further relation of pituitary to thyroid activity seems more than probable in view of the definite thyroid stimulation found by Loeb & Friedman<sup>(15)</sup> with acid extract of anterior pituitary in guinea-pigs, and a similar connexion found in guinea-pigs and dogs by Janssen & Loeser<sup>(16)</sup>. Hertz & Kraues<sup>(17)</sup> have lately been able to follow this effect to exhaustion. Furthermore, a definite seasonal variation in iodine content of thyroid taken from animals and birds has been observed by Kendall & Simon-

sen (18), Dawbarn (19), and Cruickshank (20). All these increases took place in the period of increasing daylight.

It is, however, anything but clear why the effect, if due to this cause, should be limited to those pigs born in the previous summer and autumn, since in man stimulation of the thyroid or even administration of the

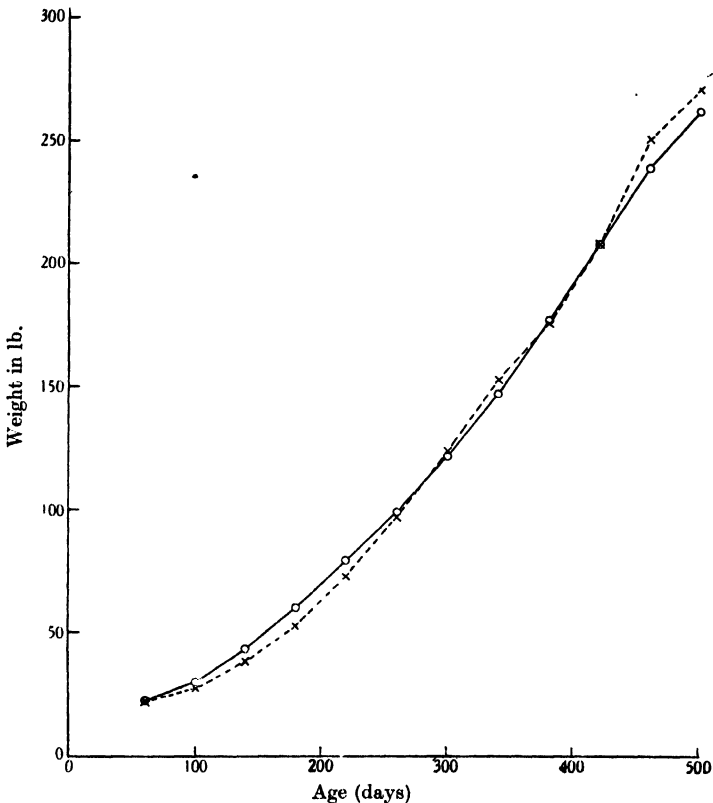


Fig. 3. Actual average live weights of pigs A-K and M (full line) compared with the average of the computed live weights.

gland *per os* is enough to cause an appreciable rise in the metabolism at all ages, and Hosker (21) finds stimulation of feather growth by thyroid feeding of Rhode Island Red fowls to take place irrespective of age. It may possibly be connected with variation of the temperature conditions in the pens which were not recorded (see Riddle *et al.* (22)). It is also noteworthy that Burton (23) concludes that the maximum metabolism per square metre per day should occur in pigs at 250 days but it is difficult to understand his association of this age with *weaning* in pigs.

## 324 *Fasting Metabolism of Various Breeds of Hog*

It may be added that, although the animals were in the dark at the time of the experiment, the after-effects of stimulation in the pens would probably be sufficient to produce a rise in metabolism since, in man at all events, the effects of thyroid stimulation do not pass off for many days after the withdrawal of the stimulus. The danger of arguing from one species of animal to another, however, is well emphasized in the literature on this matter.

Fig. 3 shows the result of a very interesting investigation on the group of pigs A-K and M. These all grew well, and were more or less contemporaneously under test. They were also subject to like conditions of housing, etc., received like rations and were in every way comparable. The continuous line in the figure shows the actual weight of the pigs (fasting) at the ages shown on the abscissa, while the dotted line shows the average weight that these same pigs should theoretically have attained at these ages, computed from the fasting metabolisms measured, allowing for the temperature at which they were kept, the nett energy of the ration, and its amount, muscular energy expenditure and the use of thermic energy surplus for maintenance of body temperature where this was available. For this computation certain assumptions had to be made which will now be discussed.

In the first place the temperatures in the animal house were not recorded, but it is known that the summer average temperature was in the neighbourhood of  $21^{\circ}\text{C}$ ., while that in winter was about  $10^{\circ}\text{C}$ . A temperature scheme was therefore assumed as follows.

July, August $21^{\circ}\text{C}$ .	April, November $14.4^{\circ}\text{C}$ .
June, September $18.8^{\circ}\text{C}$ .	March, December $12.2^{\circ}\text{C}$ .
May, October $16.6^{\circ}\text{C}$ .	February, January $10^{\circ}\text{C}$ .

This, it is felt, will represent conditions fairly closely. When the pigs were under experiment the temperature was of course accurately recorded.

The second assumption arises from the fact that no figures exist to my knowledge of the energy content of the gain in weight made by pigs fed in anything like the way ours were, i.e. on rations fixed with a view to avoiding fattening beyond that which inevitably occurs when animals are fed for maintenance and growth only.

The starting points for the assumptions made in this regard are: (a) a paper by Hammond<sup>(24)</sup> in which diagrams are given showing how the changes in body form and composition are brought about by differences in the time and rate of growth of different parts of the body (bone, muscle and fat), and (b) a paper by Edin & Helleday<sup>(25)</sup> from which the nett energy of the gain in normally fed bacon pigs

can be deduced by differentiating equation 9b.<sup>1</sup> Converted to cal. per lb. and lb. live weight, this reduces to

$$\frac{dy}{dx} = 950 + 7.6 x,$$

where  $y$  is the total nett energy content in calories and  $x$  the live weight of the pig in lb. Hence the expression on the right of the equation gives the nett energy content of the gain per lb. at live weight  $x$  lb. for bacon pigs. It is, however, clear that while this equation will hold at the beginning of our experiments, the nett energy of the gain made with our pigs must be progressively less than that determined from this equation, owing to the smaller ration fed and occasional fasts causing slower growth (see Hammond (24)). It has seemed reasonable to assume that the term in  $x$  here is determined by the fat proportion in the gain, and to allow for the fact that our pigs were on a still lower plane of nutrition than Hammond's low-nutrition animals. His curves for these pigs have been extended therefore longitudinally (along the abscissa) until the 200 lb. proportions of bone, muscle and fat are shown at 400 lb. and the dip in the fat curve has been doubled. Although these curves of Hammond's are diagrammatic they do represent the *proportions* approximately correctly, and the allowance of extra dip in the fat curve is reasonable in pigs kept on such a low plane of nutrition as ours were. The peak values for bone, muscle and fat are set as much further to the right as Hammond's low-nutrition peaks are to the right of his high-nutrition peaks.

From this diagram the ratio of muscle to fat in the gain has been read off and compared with similar readings from Hammond's diagram. When the proportion of muscle is equated to that in Hammond's diagram the fat proportion taken from the modified curves is clearly less for all readings but the initial one, which was taken as 20 lb., as we usually started with our pigs at about this weight. The figures are given in Table I.

The ratio of the figures in the last column to the figure in the third column in Table I then gives the proportion in which the coefficient of  $x$  in the equation should, on these assumptions, be reduced to give the nett energy of the gain at the weights in the first column for our pigs.

Accepting this, the values deduced for the coefficient agree with the following equation for nett energy of gain:

$$\frac{dy}{dx} = 950 + 28.5 x^{0.56} \text{ (for our pigs).}$$

<sup>1</sup> There is a misprint in the paper mentioned; this equation should clearly read  $y_{\text{therms}} = 2.089 x + 0.0189 x^2$ .



Table I. *Fat proportions in the gain from Hammond's bacon pig diagram compared with similar figures from our modified diagram*

Live weight lb.	Hammond's diagram		Modified diagram		With muscle equated to Hammond's
	Muscle	Fat	Muscle	Fat	
20	26	7	21	6	7
40	39	11	34	7	8
60	52	16	45	8	9
80	68	25	56	11	13
100	85	35	68	14	17.5
120	97	43	80	19	23
140	94	54	95	24	24
160	86	74	100	34	29
180	68	86	91	43	32
200	52	98	80	54	35

The energy expenditure on muscular work has been estimated at 50 per cent of the fasting metabolism as suggested by Wood (26).

It remains to give an example of the computation; for this we will select the period 19 October–14 December 1929, with pig B.

At the beginning of this period, i.e. at the close of experiment XII with this animal the weights were: computed from previous periods, 177.4 lb.; actual, 186 lb.

The period in question comprises 56 days, for 28 of which the ration was  $4\frac{1}{2}$  lb. per day of a mixture having a nett energy content of 1047 cal. per lb., and for the remainder of the time until the first day of experiment XIII  $5\frac{1}{4}$  lb. per day of the same mixture.

At the beginning of this period the fasting katabolism was 1.610 cal. per min. at a temperature of 16.0° C. Correcting this to critical temperature by Capstick & Wood's curve (26) we find 1.420 cal. per min. at the critical temperature.

At the close of the period the fasting katabolism was 1.870 cal. per min. at 13.0° C. which, corrected, gives 1.458 cal. per min. at the critical temperature.

We have therefore:

Between dates	Nett energy re- quirement per min. at critical temperature			Temp. °C.	Total nett energy re- quirement for sub- period at critical temp. cal.	Total actual requirement for sub- period cal.	Meta- bolizable energy in meal supplying total nett energy cal.
	Inter- polated	Mean	Days				
19–31 Oct.	1.420	1.424	13	16.6	26,650	29,550	33,350
1–30 Nov.	1.429	1.439	30	14.4	64,050	77,800	80,000
1–8 Dec.	1.451	1.451	8	12.2	16,700	22,150	20,850
9–13 Dec.	1.454	1.456	5	13.0	10,450	13,400	13,050
	1.458						

The mean nett energy requirement per min. for the sub-periods is obtained by linear interpolation (col. 3). In col. 4 the number of days in the sub-period is given, and the temperature corresponding to the month is brought in in col. 5 from the table. Col. 6, the total nett energy requirement for the whole sub-period, is obtained by multiplying the figure in col. 3 by that in col. 4, and by 1440 the number of min. in a day. The total actual requirement for the sub-period in col. 7 is obtained by correcting the critical temperature figure of col. 6 back to the temperature of col. 5, while col. 8, the metabolizable energy in the amount of meal yielding nett energy equal to the figure in col. 6, is got by multiplying this figure by  $5/4$ . The last sub-period corresponds to the time during which the pig was in the calorimeter, and the temperature is that of the instrument.

By a comparison of the figures in cols. 7 and 8, it is clear that the thermic energy included in the amount of the ration required to provide maintenance for the first two sub-periods (col. 6) is more than enough to provide for the maintenance of body temperature. In the last two periods this is not so, the total deficiency being  $1300 + 350 = 1650$  cal. This, however, may be supplied equally as nett or thermic energy, so that it corresponds to a nett energy requirement of the meal mixture  $= 1650 \times \frac{4}{5} = 1320$  cal.

Thus in the whole period the computed requirement for maintenance *in terms of nett energy* is as follows:

For maintenance (=sum of figures in col. 6)	...	117,850 cal.
For muscular movement	... ..	58,920 „
For the deficiency mentioned in the previous paragraph		1,320 „
	Total	178,090 „

Turning now to the actual nett energy supplied we have:

For the first 28 days of the period  $4\frac{1}{2}$  lb. per day at  
 $1047$  cal. per lb.  $= 28 \times 4\frac{1}{2} \times 1047$  cal.  $= 132,000$  cal.

For the remaining 24 days up to and including the first  
 day of the experiment at  $5\frac{1}{4}$  lb. per day  
 $= 24 \times 5\frac{1}{4} \times 1047$  cal.  $= 132,000$  „

Total nett energy supplied	264,000 „
Deduct maintenance	178,090 „

$\therefore$  Nett energy available for gain  $= 85,910$

## 328 *Fasting Metabolism of Various Breeds of Hog*

Now the average weight of the pig over this period was  $207\frac{1}{2}$  lb., and from the equation (p. 325) we have

$$\frac{dy}{dx} = 950 + 28.5 (207.5)^{0.56} = 1515 \text{ cal. nett energy per lb. gain;}$$

$\therefore 85,910 \text{ cal. will account for a gain of } 56.6 \text{ lb.,}$   
 The observed gain was  $43.0$  „

Hence

Weight at beginning of period:

	Computed	177.4 lb.	Observed	186 lb.
Gain	„	56.6 „	„	43 „
$\therefore$ Weight at end of period	„	234 „	„	229 „

Each separate period of each of the pigs has been computed in this manner and the average results for the eleven pigs are shown as stated in Fig. 3.

Although the average curves for the eleven pigs agree so remarkably well with theory, it must be emphasized that the curves for the individual pigs showed no such agreement, the differences between the observed and computed results being in some cases quite large, in one case over 30 per cent of their mean. This gives further point to the opinion expressed by the writer in a previous paper (7) that nett energies should be regarded as statistical rather than physiological constants. In regard to this view and the arguments with which it is supported a recent publication by Odriozola (27) in which he adopts this view as the result of practical feeding and slaughter experiments is of interest. Further point is given to the writer's views on this matter by the work of Mitchell *et al.* (28, 29) who find the nett energy in cattle and fowls to decrease considerably with a rise in the plane of nutrition, and the views expressed recently by Forbes (30, 31, 32) who states that "net energy values of individual foodstuffs are fundamentally variable and hence are not practical standard measures of reference". Forbes wishes to substitute the nett energy of the nutritively complete ration as a conventional measure and this may well be possible as a matter of practical convenience.

Before leaving the question of this computation it should be remarked that in those cases where a considerable difference develops between the total computed and the total actual weight there seems no proper reason for selecting either of these for the computation of the nett energy content of a pound gain. If the first be selected and if it is the higher of

the two the estimated figure will almost certainly be too large, while if the second is selected it will probably be too small, since the animal is old for its weight by comparison with the mean. Hence in these cases a correction is applied to bring the result to the mean of the computed average and the actual average for the period. This correction is cumulative from period to period, but does not make more than two or three pounds difference even in extreme cases.

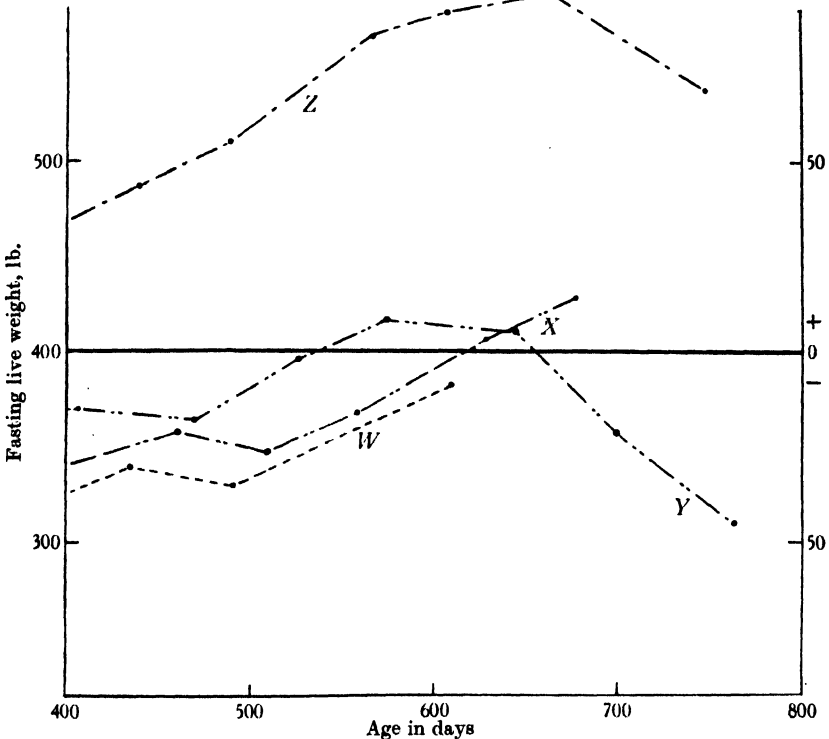


Fig. 4. Further progress of pigs W-Z (subsidiary to Fig. 1 of the first paper of this series, *J. agric. Sci.* (1934), 24, 326.

These computations have been made for all periods with all the pigs. In the case of pigs N-V which, as remarked in Paper I of this series, did not grow as well as those discussed above, owing partly to the lower winter temperatures and lack of exercise with troubles resulting therefrom, there is no agreement between the average curves as in Fig. 3. This lack of agreement was probably accentuated by the method of computation, since the curve deduced from those of Hammond could hardly be expected to apply. Pigs W-Z received larger amounts of food to

compensate for the lower winter temperatures (see Paper I). The deviation of live weight from the mean of pigs A-M is shown in Fig. 4 as in Fig. 1 of the paper quoted. It is clear that this small addition to the ration was sufficient to prevent the falling away of the growth curve as in pigs N-V, in pig Z it was clearly *more* than sufficient for the purpose. Hence, while it would probably be going too far to say without further evidence that the falling away in pigs N-V was entirely due to the low temperature in winter rendering the ration insufficient, there is *prima facie* evidence that this was at least an important factor. The Fig. 4 completes Fig. 1 in Paper I of the series for these four pigs.

Despite the agreement as regards growth, the computations of gain made on the foregoing plan are not very satisfactory for two reasons: (1) four pigs is too small a number to give a satisfactory average curve, and (2) all these pigs suffered unavoidably from lack of proper exercise, and as constipation was a frequent trouble with them this may well have caused a fall in the digestibility of the ration and consequently in its nett energy content per lb. If the nett energy is taken as 980 cal. per lb. instead of 1047 cal. per lb., the agreement is quite as good as can be expected with only four pigs to average. A reduction in digestibility of this magnitude is not an unreasonable supposition.

The question of how far the present results justify the hypotheses as to rationing, made in a previous publication<sup>(7)</sup> mentioned, must be deferred for the present.

#### SUMMARY

From metabolism experiments carried out on the pigs enumerated in the first paper of this series the following conclusions are drawn:

(a) The metabolism in a state of inanition appears to be more a function of a power of the weight than of the true surface area as determined by the photographic method.

(b) Hogs born in the summer and autumn of one year appear to show two maxima of metabolism; one almost immediately, as has been observed previously, and another during the following summer, provisionally ascribed to the effect of light on thyroid activity, produced by the intermediary action of the anterior pituitary.

(c) Actual, and theoretically computed average growths are shown to agree remarkably well in the case of eleven of the pigs which were treated in an exactly similar manner. Individual growth curves showed no such agreement. This is considered to strengthen the evidence for the writer's previously stated view that nett energy is a statistical rather than a physiological constant.

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# INVESTIGATIONS ON THE ROOT NODULE BACTERIA OF LEGUMINOUS PLANTS

## XIX. INFLUENCE OF VARIOUS FACTORS ON THE EXCRETION OF NITROGENOUS COMPOUNDS FROM THE NODULES

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(With Plate III and One Text-figure)

DURING the course of our investigations on the excretion of nitrogenous compounds from the nodules of leguminous plants we have often noted that the rate of excretion varies considerably in different experiments, without any apparent reason for such variations. We have earlier described some of the experiments in this paper, and suggested that the air content of the medium is one of the factors which influences the extent of excretion. However, according to subsequent work, the air content of the medium influences largely the actual process of nitrogen fixation, while it has no specific effect on the rate of excretion. Continued research on the nature of excretion and its dependence on various factors has led to certain observations which throw new light on this theoretically and practically significant problem. Our results up to the present are described below.

### GENERAL CONSIDERATIONS

Exact study of the excretion of the nitrogen compounds necessitates the use of a sterile culture system<sup>1</sup> in which the legume is inoculated with a pure culture of the appropriate nodule organism. Under such conditions the free-living nodule bacteria do not fix nitrogen and, since the medium contains no foreign micro-organisms, any accumulation of nitrogenous substances in the medium must presumably be a result of

<sup>1</sup> We have used the term "sterile culture system" for our cultures even in cases where the leguminous species is inoculated with the appropriate nodule organism. The term "sterilized culture" would perhaps be more appropriate, although even this term is somewhat inaccurate, since it does not denote that contamination by foreign organisms is excluded throughout the experimental period.

the action of the nodules. We have earlier shown that the excreted nitrogen compounds consist mainly of *l*-aspartic acid and some other amino acid, which is precipitated by phosphotungstic acid, and very little oximes (Virtanen & Laine, 1935, 1936). Furthermore, we have now also shown that similar excretion of amino acids does not occur from the roots of uninoculated legumes, growing on nitrate or ammonia nitrogen. Hence it follows that the excretion must take place from the nodule bacteria, probably from the intranodular ones, and not from the roots.<sup>1</sup>

The following facts suggest that the excreted amino acids are direct products of nitrogen fixation rather than the results of protein decomposition:

(1) Excretion of the nitrogenous compounds begins immediately upon the appearance of the nodules and proceeds at a maximum rate in young nodules, before the blooming stage of the plant. Macroscopical and microscopical examinations show that at this stage the nodule tissue is perfectly sound, without any signs of decay.

(2) Should the excretion be a result of protein decomposition, it would be difficult to explain why only two amino acids are excreted. It should be remembered that the nodule proteins, like proteins in general, contain several different amino acids, including also aromatic compounds (Virtanen & Torniainen, 1936), so that a breakdown of the nodule proteins would be expected to cause an excretion of numerous different amino acids.

It may be mentioned here that Bond (1936) has determined the rate of transfer of fixed nitrogen from the bacteria to the host over successive periods in the life cycle of the latter, and likewise concludes that a very high proportion of the nitrogen fixed by the bacteria is regularly liberated without appreciable delay into the host cytoplasm. Bond's results are also in perfect accord with the view that the legumes receive their nitrogen nutrition from the nodules in the form of amino acids, as postulated by us several years ago.

<sup>1</sup> It may also be mentioned here that we have shown new, incontestable evidence for the fact that the excretion of amino acids occurs from the nodule bacteria and not from the roots. This was demonstrated with quartz sand cultures of peas using our sterile culture system. A sand-filled glass tube was sunk into the quartz sand so that its neck extended above the surface of the sand in the culture flask. The sand in the tube alone was then inoculated with the nodule organism. Nodulation therefore naturally occurred only in the roots which grew into the small glass tube. Under these conditions amino acids were excreted only into the sand contained in the tube and not into the sand in the culture flask outside the tube, where the major part of the roots grew.



## EXPERIMENTAL

The experimental technique employed in this laboratory has been described by us earlier in a German paper (Virtanen *et al.* 1933). However, since this technique has been subsequently improved to some extent, it might be appropriate to describe it here in detail, particularly as this has not been previously done in English papers.

The chief feature of the technique is that all manipulations are carried out as aseptically as possible, using a special room where the contaminating micro-organisms could be destroyed with a quartz mercury lamp. This made it possible to eliminate cases of contamination almost completely.

A desired number of fully ripe and faultless seeds of equal size with absolute alcohol were placed in the suction flask (*a*) of the sterilization apparatus for seeds, illustrated by Plate III, fig. 1. The seeds were shaken in the alcohol for 2 min., after which the alcohol was poured off through the side tube of the suction flask. The seeds were next treated with sublimate solution (1 : 1000), which was poured out from the side flask (*b*). Seeds of cereals were shaken in the sublimate solution for 4 min., and peas from 4 to 10 min. The sublimate solution was then drained off through the side tube of the suction flask, and the seeds were repeatedly washed with sterile, distilled water from the other reserve flask (*c*). The seeds were left in a suitable quantity of the last washing water, where they were allowed to swell for about 24 hours.

The swollen seeds were transferred with the aid of sterile forceps into a test-tube containing 5 ml. of sterile agar (2 per cent agar in distilled water). One seed was placed on the surface of the agar in each tube. Any contamination was easily detected, since in such cases the micro-organisms produced visible colonies on the agar. When the seedling had almost reached the cotton plug of the test-tube its root was inoculated with a loopful of the pure culture of the specific nodule organism.

The culture vessel consisted of a suction flask, or a three-necked Woulff's bottle. The latter was particularly suited for associate culture experiments. The legume grew up through one neck, the non-legume through another. The culture flasks were stoppered with plugs of rolled cotton (see Plate III, fig. 2), and connected with the reserve flask by means of a rubber tube.

Before autoclaving, part of the nutrient solution was added to the quartz sand in the culture flask, the major part being diluted with distilled water and poured into the reserve flask. During the growth period

sufficient nutrient solution was then added to the sand from the reserve flask to keep the sand just moist. The plants generally consumed the entire amount of nutrient solution so that they also actually receive the nutrients originally dissolved in the solution.

The culture flasks with the nutrient media were autoclaved for 20 min. at 120° C. After cooling, they were placed in the sterilized room on a steel table. The table was flooded with alcohol which was burnt off. The cotton plug of the flask was removed with sterile pincers, and spread out on the freshly sterilized table. The inoculated seedlings were taken out of the test-tubes with the pincers, and placed on the cotton. The cotton was then rapidly rolled around the plants with the aid of the pincers, whereupon it was placed in the neck of the culture flask so that the roots of the plant came down into the nutrient medium and the stalk could grow out through the cotton plug into the open air. Prior to the transfer of the plant the neck of the culture flask with the protruding cotton plug was protected by an inverted beaker.

At the conclusion of the experiment, the sterility of the medium was tested bacteriologically in milk, and in meat extract-peptone gelatin.

The bacteriological controls were observed during 14 days. During the course of the experiment any contamination of the cultures could be easily detected macroscopically, since in such cases the roots appeared slimy and dark coloured, and the plants soon began to wither.

The composition of the nutrient solution was:  $\text{Ca}_3(\text{PO}_4)_2$  0.25 g.,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  0.25 g.,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.394 g.,  $\text{KCl}$  0.25 g.,  $\text{FeCl}_3$  (5 per cent solution) 3 drops. Distilled water to 1000 ml.

In part of the experiments the nitrogen determinations were made on three 100 g. samples of sand according to Kjeldahl. The total quantity of nitrogen in the sand was then calculated from the mean value of the three determinations. The deviations between the different determinations did not exceed 10 per cent. In other experiments we employed the improved method (Method 2) described earlier (Virtanen & v. Hausen, 1935). This method renders it possible to make the nitrogen determinations on a composite sample of 1200 g. sand.<sup>1</sup> The method employed in each different case has been mentioned in the text.

Under sterile conditions, the nitrogen content of the medium of the uninoculated cultures cannot increase. This fact has been established conclusively in numerous experiments. The amounts of nitrogen found in the control sands are identical with those initially present in the sand. The nitrogen content of our quartz sand has varied in different batches from 2.6 to 3.7 mg. per kg. of dry sand. The chemical nature of this

nitrogen has not been investigated. It is important to note that peas cannot utilize this nitrogen. The nitrogen content of the control peas is practically equal to that found in the seeds. In the variety of peas employed the nitrogen content of the seeds averaged 7 mg. per seed.

If no controls are run, then it is necessary to subtract from the values obtained for the inoculated cultures the amounts of nitrogen initially present in the seeds and sand respectively. As already mentioned, the values for the controls are practically equal to these subtractions. Control experiments are necessary in associated culture experiments, since barley, for instance, seems to take up some of the nitrogen present in the sand.

## RESULTS

In our earlier papers on the nitrogen excretion we have assumed that the excretion takes place from the root nodules. However, this assumption was not proved experimentally, and it was therefore possible that the excreted compounds might originate from the roots. After having ascertained that the excreted nitrogen compounds consist of amino acids, it became possible for us to settle the point definitely. Should the excretion take place from the roots (see Bjälfve, 1935), it would then be expected that similar amino acids were excreted also from the roots of uninoculated legumes growing on mineral nitrogen. Table I shows clearly that this is not the case.

Table I

(1) *Inoculated peas, no nitrogenous fertilization*; 3 l. Woulff's bottle; 4 kg. quartz sand; 2 l. nutrient solution. Two pea plants, inoculated with strain H X. Period of growth: 2 Nov.-9 Dec. 1935.

(2) *Uninoculated peas, growing on nitrate nitrogen*. Duplicate experiment. Otherwise as in (1).

(3) *Controls, uninoculated peas, no nitrogenous fertilization*; otherwise as in (2).

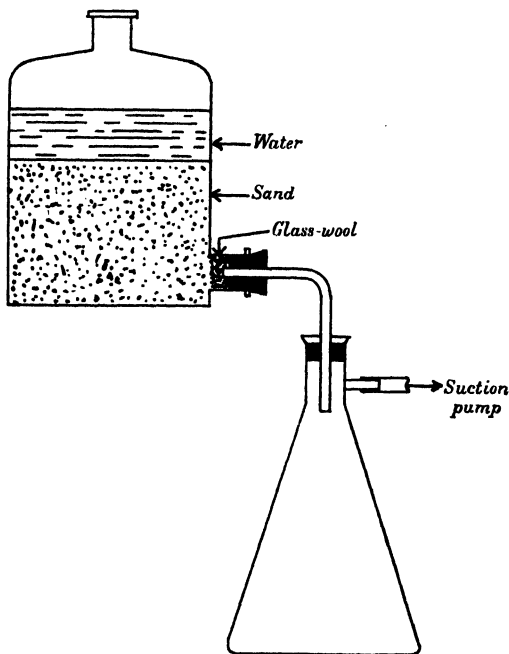
Initial nitrogen content of the sand 3.7 mg. per kg. dry sand.

No. of exp.	Dry weight of 2 plants, g.	N mg.		Extracted N		Amino N, % of extracted N	Extracted amino N, mg. per 2 plants
		Plants	Sand	Mg.	% of N in sand		
1	4.867	113.4	136.8*	94.3	72	95.4	90.0
2 (mean)	4.125	94.6	—	—	—	—	3.9
3	0.490	12.4	15.2	—	—	—	1.7

\* After subtraction of the control.

In all experiments the extraction of the sand was carried out in exactly the same manner. The extraction vessel was a 10 l. flask, provided with an outlet tube near the bottom (see Text-fig. 1). The outlet tube was

filled with spun glass to filter off the sand, and connected by a bent glass tube with the suction flask. The sand was carefully sifted to remove root particles, and extracted with acidified water (sulphuric acid to pH 4). For each 4 kg. of sand 1.5 l. of water were used. The mixture of sand and water was thoroughly shaken, and the water then drained off with suction. The sand was washed 10 times, the total amount of water used being thus 15 l. The solution was concentrated under reduced pressure at 40° C. to a volume of 100 ml.



Text-fig. 1. Showing how the sand was extracted.

Total nitrogen was determined from the extract by the official Kjeldahl method, and amino nitrogen according to van Slyke. In the latter determination, the time of reaction was extended to 30 min., since we found that otherwise all amino groups will not react completely.

It will be seen from Table I that 130.8 mg. nitrogen was excreted into the sand in the culture of inoculated peas. This corresponds to 56 per cent of the total fixed nitrogen. With the method of extraction employed, 72 per cent of the nitrogen content of the sand could be recovered in the extract. Of this amount, 90 mg. or 95.4 per cent was amino nitrogen. In the corresponding experiment with uninoculated peas growing on nitrate

nitrogen, only 3.9 mg. of amino nitrogen could be extracted from the sand. In view of the fact that the corresponding figure in the control experiment was 1.7 mg., it can be concluded that practically no excretion had taken place from the roots. The excretion is thus a function of the nodules.

We have earlier shown that no appreciable excretion occurs in water cultures of inoculated legumes, although the plant grows excellently, especially when air is bubbled through the medium (Virtanen & v. Hausen, 1936). This observation led us to the conclusion that the nodules must be in direct contact with solid materials in order that excretion may occur. Subsequently we made experiments with different materials in order to ascertain the physical nature which the solid materials must possess to bring about a distinct excretion.

In some experiments with water cultures the flasks were filled with glass beads. In order to supply sufficient air to the roots the cultures were aerated for several hours a day. There was ample nodulation but no distinct excretion of nitrogen. In view of the fact that a powerful excretion occurs in quartz sand cultures, it became apparent that only absorptive materials with a large surface area could promote the excretion. We therefore made new experiments by adding kaolin or finely ground cellulose to the medium. The results of these experiments are summarized in Tables II-III.

A distinct excretion has thus occurred also in experiments where the medium consisted of cellulose or kaolin. However, the extent of excretion was much smaller with cellulose than with kaolin or sand. This

Table II

*Exp. 1.* 1 l. suction flask, 325 g. well washed nitrogen-free  $\alpha$ -cellulose. Two pea plants, inoculated with strain H X. 1 l. nitrogen-free nutrient solution. Period of growth: 27 Dec. 1935-10 Feb. 1936. Artificial illumination. After harvesting, the cellulose was washed three times with 500 ml. water (pH 4), whereby all excreted nitrogen compounds passed into solution. The last washing liquid contained practically no nitrogen. The extract was evaporated to a volume of 100 ml. Nitrogen was determined according to Kjeldahl.

*Exp. 2.* 3 l. Woulff's bottle; 800 g.  $\alpha$ -cellulose. Two peas, inoculated with H X. Nitrogen-free nutrient solution (as in Exp. (1)). Period of growth: 27 April-18 June 1936 (natural illumination). After harvesting, the cellulose was washed three times with 1 l. water (pH 4).

*Exp. 3.* As in Exp. 2.

No. of exp.	Dry weight g.	N mg.		Total fixed N mg.	Excreted N, % of total fixed N
		Plants*	Cellulose		
1	3.364	61.8	11.3	73.1	15.5
2	5.733	108.0	15.4	123.4	12.5
3	7.661	167.1	18.2	185.3	9.8

\* After subtraction of the nitrogen in seeds.

Table III

2 l. suction flasks; 1.3 kg. nitrogen-free kaolin; 1.5 l. nitrogen-free nutrient solution. Two peas, inoculated with H X. Period of growth: 21 July–18 Sept. 1936. The kaolin was mixed with 3 l. water, the roots were carefully removed and the mixture was acidified with sulphuric acid to pH 4 and centrifuged clear. The solution was evaporated in a vacuum and analysed for nitrogen. The kaolin was first dried at 50°, whereupon two samples of 13 g. were analysed according to Kjeldahl.

Dry weight g.	N mg.			Total excreted N	
	Plants*	Kaolin†	Solution	mg.	% of total fixed N
5.748	118.9	42.0	8.8	50.8	30

\* After subtraction of the nitrogen in seeds.

† After subtraction of the blanks on kaolin.

is probably due to the fact that both kaolin and sand absorb the nitrogen compounds in question much more firmly than does cellulose. Thus, a quantitative extraction of the nitrogen compounds from the cellulose was accomplished by washing 800 g. of cellulose three times with a total quantity of 3 l. water, the last washing liquid being practically nitrogen-free, whereas only about 80 per cent of the excreted nitrogen compounds could be extracted from 4 kg. of the quartz sand, by washing the latter with water 100 times, with a total volume of 40 l. If a given amount of cellulose is washed with an equal quantity of water, the major part of the nitrogen compounds present in it will pass into solution, while only about 10 per cent is obtained in solution by a similar treatment of quartz sand. A quantitative extraction of the nitrogen compounds from the sand could not be accomplished so far. The experimental results recorded above thus indicated that *the extent of excretion depends on the ability of the medium to absorb the nitrogen compounds formed in the nodules.*

Our experience of the excretion in different kinds of media led us to the assumption that the distribution of the nitrogen compounds between the nodule and the external medium is determined by an equilibrium which lies strongly on the side of the nodule. Consequently, only negligible amounts of nitrogen are passed into the medium in water cultures. Addition to the medium of materials which absorb the excreted nitrogen compounds shifts this equilibrium more to the side of the medium and causes a continuous excretion.

On this assumption it would be expected that:

(1) Within reasonable limits, the extent of excretion should be the greater, the higher the quantity of absorbent solid materials in the medium. Our earlier work with culture flasks of varying size and with

different quantities of quartz sand, are indeed in accord with this postulate (Virtanen & v. Hausen, 1935). Thus in an experiment with peas we found the following amounts of excreted nitrogen in the sand (1 and 2 l. flasks start of flowering; 3 l. flask before flowering):

	1 l. flask 1.3 kg. sand*	2 l. flask 2.7 kg. sand*	3 l. flask 3.4 kg. sand†
N in plants, mg.	111.9	78.8	50.0
N in sand, mg.	33.6	75.4	66.8

\* Four plants.

† Three plants.

Similarly, in another experiment with varying amounts of sand in flasks of equal size, we obtained the following results (Virtanen & v. Hausen, 1936, p. 286):

	3 l. flask 1.5 kg. sand	3 l. flask 4.9 kg. sand
N in plants, mg.	127.7	63.7
N in sand, mg.	45.2	75.2

We have earlier assumed these results to be ascribable to the effect of an increasing air content in the medium. It seems, however, that another important reason is the increasing absorption by increasing amounts of sand.

However, it should be noted that in one of the above experiments (3 l. flask, 1.5 kg. sand) only the tips of the roots were embedded in the sand, while in the other culture (4.9 kg. sand) the roots were entirely embedded. In the latter case all nodules were thus surrounded by the absorbing sand, while in the former case a great number of nodules grew in the air. It is therefore also possible that the differences in the extent of excretion were due, at least in part, to the difference in the number of nodules which were in direct contact with the sand.

Table IV

3 l. Woulff's bottles, 3.4 kg. quartz sand; 2 l. nutrient solution. One pea + one barley in each bottle. Period of growth: 2 May–9 June 1932. Initial nitrogen content of the sand 2.7 mg. per kg.

No. of culture	Inoculation	Dry weight g.		N mg.*			Total fixed N mg.	Excreted N, % of total fixed N
		Pea	Barley	Pea	Barley	Sand		
1	Strain H IV	1.462	1.791	41.8	31.6	89.0	162.4	74.3
2	"	2.048	1.660	55.8	21.7	77.4	154.9	64.0
3	"	1.382	1.432	42.6	24.4	93.9	160.9	73.5
4	"	2.400	0.655	60.7	13.0	67.6	141.3	57.0
5	None; control	0.297	0.063	6.4	0.7	10.0†	—	—

\* The values for the inoculated cultures are given after subtraction of the corresponding values for the control.

† In the earlier report, published in the *Biochem. Z.*, this figure is erroneously given as 1.0 mg.—a printer's error.

(2) The extent of excretion should be greater when the legume is grown in association with some non-legume, whose roots continually absorb the nitrogen compounds excreted from the nodules, than when the legume grows alone. Numerous experiments in this laboratory show that this is indeed the case. (A report of these experiments will appear shortly in this *Journal*.) Table IV shows the extent of excretion in associated cultures. The term, excreted nitrogen, indicates the sum of nitrogen present in the barley and in the sand.

In corresponding experiments with two peas alone in each flask, the extent of excretion was proportionally lower, as evidenced by Table V.

Table V

3 l. Woulff's bottles, 3-4 kg. quartz sand; 2 l. nutrient solution. Two peas in each flask. Period of growth: 2 May-9 June 1932.

No. of culture	Inoculation	Dry weight g.	N mg.*		Total fixed N mg.	Excreted N, % of total fixed N
			Peas	Sand		
1	Strain H IV	2.564	74.5	79.4	153.9	51.6
2	"	4.644	130.1	87.2	217.3	40.1
3	"	5.788	138.9	91.3	230.2	39.7
4	"	5.225	123.4	96.7	220.1	44.0
5	None; control	0.512	14.4			

\* The values for the inoculated cultures are given after subtraction of the corresponding values for the control.

(3) If the plant grows in a dry atmosphere, when the rate of transpiration is high and fresh watering solution must be continuously added to the sand, it would be expected that part of the nitrogen compounds were dissolved in the water, to be subsequently taken up by the roots. Under such conditions the final nitrogen content of the sand should be lower than when the humidity of the atmosphere is high and the rate of transpiration lower, so that very little, if any, watering solution need be used. In order to settle this point we carried out parallel experiments in which part of the cultures were grown in the greenhouse where the percentage humidity varied from 40 to 50 per cent, while the other cultures were placed in a box of U-glass (200 × 100 × 50 cm.), where the percentage humidity was maintained at 85-90 by passing a continuous stream of water along the back wall of the box. The results are compiled in Table VI.

In both series the growth of the plants was about equal, whereas the final nitrogen content of the sand in series I was more than double that in series II. The excretion was fairly weak in both series, apparently due in part to the small size of the culture flasks, and the small quantity



## 342 *Root Nodule Bacteria of Leguminous Plants*

of sand. The difference between the two series is, however, quite distinct, and supports the view expressed above.

Table VI

1 l. suction flasks, 1.3 kg. quartz sand; 1 l. nutrient solution. Two inoculated peas in each flask. Period of growth 2 May–19 June 1936. Harvested in full bloom.

% humidity	No. of cul- ture	Dry weight g.	N mg.*		Total fixed N, mg.	% of total fixed N in sand	Water added from the reserve flask ml.
			Plants	Sand			
I							
80–90	1	3.733	91.3	16.8	108.1	15.5	0
80–90	2	2.846	65.7	17.9	83.6	21.4	0
80–90	3	3.191	74.1	14.9	89.0	16.1	0
80–90	4	2.446	69.4	13.2	82.6	16.0	0
II							
40–50	5	3.415	78.3	3.2	81.5	3.9	500
40–50	6	3.572	88.3	7.6	95.9	8.0	580
40–50	7	2.492	58.0	9.1	67.1	13.6	420
40–50	8	3.698	82.2	5.0	87.2	5.7	630

\* After subtraction of the controls (14.3 mg. in peas and 4.5 mg. in sand).

(4) On the assumption that there is an equilibrium governing the distribution of the nitrogen compounds between the nodule and the medium, it would be expected that the amount of excreted nitrogen would increase also in liquid cultures with increasing quantities of water. This question was studied with water cultures of inoculated peas, using a culture vessel from which the liquid could be drained off through a glass tube near the bottom of the flask. The flask was then refilled with the fresh nutrient solution. The results of one series of such experiments are illustrated by Table VII.

Table VII

1.5 l. suction flasks; water cultures; two peas in each flask. The plants were first allowed to grow in the same liquid (1 l. of solution) for 7 days, after which the nutrient solution was changed every third day. The cultures were aerated daily for 1 hour. Period of growth: 7 July–25 Aug. 1936. A total of 12 l. of the nutrient solution was used for each culture. The drained solution was immediately acidified, and evaporated to a small volume. The combined concentrates were then analysed for nitrogen.

No. of culture	Dry weight g.	N mg.		Excreted N, % of total fixed N
		Plants	Nutrient solution	
1	2.180	39.3	5.5	12.3
2	2.864	58.8	4.5	7.1

In experiments where the nutrient solution is not changed during the growth period, the amount of excreted nitrogen generally averages only from 1 to 2 mg. per culture. It seems, therefore, that an increased excretion, although a weak one, took place in the above experiments.

The experimental results so far recorded thus support our assumption that the distribution of nitrogen between the nodule and the medium is determined by an equilibrium. This equilibrium is naturally a very complicated one, involving, as it does, also the living nodule tissue. It is therefore also probable that the quantitative aspects of the equilibrium may vary considerably, depending on various factors, such as the ability of the host plant to utilize the nitrogenous compounds fixed in the nodule, etc.

This view is supported by our observation that the extent of excretion depends largely on the bacterial strain used for inoculation. Table VIII illustrates one of our trials in which each culture was inoculated with a different bacterial strain but otherwise kept under exactly similar conditions. The trial was carried out during the dark season, using artificial illumination, and the plants were harvested at the early flowering stage. These facts account for the low yields of dry matter.

Table VIII

2 l. suction flasks, 3 kg. quartz sand; 1.5 l. nutrient solution. Two peas in each flask. Period of growth: 30 Dec. 1935–27 Jan. 1936. Nitrogen determined according to method 2.

Inoculation	Dry weight g.	N mg.*		Total fixed N mg.	Excreted N, % of total fixed N
		Plants	Sand		
Strain H 30	1.697	52.9	18.9	71.8	26.3
„ H 31	1.119	19.7	18.7	38.4	48.6
„ H 32	1.387	23.4	22.9	46.3	49.5
„ H 33	1.222	21.1	31.3	52.4	59.8
„ H 34	1.232	21.7	6.3	28.0	22.5
„ H 35	1.597	46.2	58.8	105.0	56.0
„ H 36	1.362	46.1	33.6	79.7	42.2
„ H 37	1.122	21.0	30.0	51.0	58.8
„ P. Polen	1.758	52.1	8.4	60.5	13.9

\* After subtraction of the corresponding values for the uninoculated controls.

Table VIII shows that the extent of excretion (excreted nitrogen as percentage of the total fixed nitrogen) is generally rather high. It will be remembered that the rate of excretion is highest with young plants (Virtanen & v. Hausen, 1935). It is seen, however, that the extent of excretion has varied greatly with the different bacterial strains, and that it is by no means proportional to the effectiveness of the strains. Thus, for example, the plants inoculated with strains H 30 and P. Polen contained practically equal amounts of nitrogen (52.9 and 52.1 mg., respectively) whereas in the former case the percentage excretion was 26.3 and in the latter only 13.9. In the experiments with strains H 35 and H 36, the peas contained 46.2 and 46.1 mg. nitrogen, respectively, the corresponding figures for the percentage of excretion being 56.0 and 42.2. The plants inoculated with the weakest strains—H 31, 32, 33, 34 and 37—

contained about the same amounts of nitrogen (from 19.7 to 23.4 mg.) whereas the percentage excretion varied from 22.5 to 59.8. *The effectiveness of different bacterial strains in fixing nitrogen should thus not be measured by the nitrogen content of the crops alone, but also by the extent of excretion.*

The reasons why the extent of excretion varies so greatly with different strains are still unknown. It should be mentioned, however, that differences can also be noted in the excretion of nitrogenous compounds by different strains of the free living nitrogen fixer, *Azotobacter*, which also, according to our latest investigations, excretes aspartic acid.

There are also other factors which influence the excretion; of such factors, we have paid attention to the effect of nitrates. It is well known that a heavy nitrate dressing depresses the function of the nodule bacteria, and possibly also the nitrogen excretion. However, it is not known whether very small amounts of nitrate would similarly influence these processes, particularly the excretion. Since this question is of considerable importance in practical farming a closer study was held desirable. The excretion of nitrogen compounds can be quantitatively determined even in sand cultures containing nitrates, since the excreted compounds consist almost exclusively of amino acids, and are extractable with water. The extent of excretion can thus be determined by analysing the amino nitrogen content of the extract according to van Slyke.

Table IX. *Excretion in the presence of nitrates*

2 l. suction flasks; 3 kg. quartz sand; 1.5 l. nutrient solution. Each flask contained two peas, inoculated with strain H 20. Period of growth 26 Feb.-17 Apr. 1936. Two cultures (four plants) were used for each determination. After harvesting, the root particles were removed from the sand which was then washed ten times, each time with 2 l. of acidified water. The total volume of water was thus 20 l. The extract was evaporated to a volume of 100 ml., and analysed for amino nitrogen. According to our experience the above method of extraction removes about 70 per cent of the total quantity of nitrogen present in the sand. The total excreted nitrogen has been calculated on the basis of this figure.

NO <sub>3</sub> -N per 4 plants mg.	Dry weight g.	N in plants mg.*	Amino-N in the extract mg.	Excreted N mg.	Total fixed N mg.	Excreted N, of total fixed N
0	4.397	94.1	33.6	48	142.1	32.4
20	4.219	96.1	8.4	12	108.1	11.1

\* After subtraction of the N in seeds.

So far we have only made one experiment in which the effect of small quantities of nitrate on the excretion was examined. The results were very clear and we therefore record them here, although we feel that further work is needed to give the results definiteness.

The excretion thus seems to be greatly lowered by even small amounts of nitrate. In the above experiment the plants were given only about 20 per cent of the total nitrogen present in the crop, while the nodules supplied the remaining 80 per cent. This light dressing with nitrate has nevertheless reduced the excretion from 32.4 per cent (in the nitrate-free cultures) to 11.1 per cent (of the total fixed nitrogen). Hence the excretion of nitrogen seems to be more susceptible to nitrate fertilization than is the nitrogen fixation. According to the above observation it might be expected that, in nitrate-rich soils, the rate of excretion would be low. Further work on this problem is in progress.

#### DISCUSSION

After having shown that the nitrogen compounds which appear in the medium in inoculated, but otherwise sterile, quartz sand cultures of legumes, consist almost exclusively of amino acids—the oxime nitrogen amounting generally to only 1 or 2 per cent of the total excreted nitrogen—we showed experimentally that the excretion is a function of the nodule bacteria, probably of the intranodular ones, and not of the roots. Other work then led to the conclusion that there exists an equilibrium determining the distribution of the nitrogen compounds between the nodule and the surrounding medium. The process is complicated by the fact that part of the nitrogen compounds are transferred from the nodule to the host plant. This would explain the variations in the extent of excretion frequently noted even between different cultures inoculated with the same strain, and growing under exactly the same experimental conditions. The above assumption is supported by our observations showing that the extent of excretion depends largely on the properties of the medium. If the medium absorbs the excreted nitrogen compounds, as is the case with cellulose, kaolin, sand and soil media, a distinct excretion occurs; otherwise, the extent of excretion is negligible (water cultures). Even with water cultures it has been found possible after repeated renewal of the liquid to demonstrate a passage of nitrogen compounds into the liquid medium.

The extent of nitrogen fixation is not determined merely by the growth and nitrogen content of the host plant, it is necessary to consider also the amount of nitrogen excreted into the medium. We use the term *total fixed nitrogen* for the total amount of nitrogen given up by the nodules to the host plant and the medium. The term *extent of excretion*, that is, the amount of excreted nitrogen as a percentage of the total nitrogen fixed, indicates the quantitative aspects of the excretion.

In associated cultures of legumes and non-legumes the total fixed nitrogen is equal to the nitrogen taken up from the nodules by the host plant *plus* the nitrogen excreted from the nodules into the medium and taken up by the non-legume *plus* the excreted nitrogen still present in the medium. The extent of excretion is thus equal to the non-legume nitrogen *plus* the nitrogen found in the medium, the sum being calculated as a percentage of the total fixed nitrogen. The extent of excretion, as thus defined, seems to rise higher in associated cultures, where the non-legume continuously absorbs the excreted nitrogen. The extent of excretion is largely dependent on the bacterial strain used for inoculation, presumably on account of differences in the structure of the nodule tissues, although experimental data are not available at present to support the latter assumption.

The rate of excretion further depends on the rate of transpiration, i.e. the amount of watering solution used. With large amounts of watering solution, excreted nitrogen compounds are extracted from the sand and are taken up by the plants through the roots. This causes a fall in the extent of excretion.

Our earlier observation that the excretion depends on the size of the culture flask and the amount of sand used, is evidently to be explained at least in part by the increased ability of larger quantities of sand to absorb more of the excreted nitrogen compounds. Another explanation for the increase of excretion with larger quantities of sand is that the root system is more extensive so that more nodules will be formed. Further research is needed to settle this point. The air content of the medium decisively influences the function of the nodule, but has apparently no specific effect on the extent of excretion.

Since legumes utilize aspartic acid readily it must be concluded that they themselves take up excreted nitrogen compounds, particularly during later stages when the activity of the nodules becomes weaker. In ordinary non-sterile cultures, where the amino acids are decomposed by other bacteria, the legumes naturally may utilize the breakdown products thus formed. It is very interesting to note that the legumes probably take up part of the fixed nitrogen through their roots, especially as this will explain the frequently noted injurious effect of non-legumes on the growth of legumes in associated cultures. Our work on the associated cultures will be described in another communication.

## SUMMARY

1. It has been shown experimentally that the excretion of nitrogen noted by us in cultures of inoculated legumes takes place from the nodule bacteria, probably from the intranodular ones, and not from the roots. No excretion of amino acids occurs in cultures of uninoculated legumes growing on nitrate nitrogen.

2. Our earlier hypothesis that the legumes receive their nitrogen nutrition from the nodules in the form of organic nitrogen compounds, particularly amino acids, is in perfect accord with our new observations concerning the process of excretion. All facts indicate that the amino acids concerned are primary products of the nitrogen fixation, and not breakdown products of proteins. Bond's valuable work along quite different lines produced results which support this conclusion. He, however, did not study the chemical nature of the nitrogen compounds in question.

3. The excretion of nitrogen occurs in media capable of absorbing the excreted nitrogen compounds (cellulose, kaolin, sand, soil). The demonstration of the excretion is not possible in water cultures except when very large quantities of water are used. On the basis of these facts a hypothesis is advanced to explain the nature of the excretion.

4. The term *total fixed nitrogen* has been used as an expression for the extent of nitrogen fixation, while the term *extent of excretion* is employed to indicate that percentage of the total fixed nitrogen which is excreted from the nodules.

5. The extent of excretion depends largely on the strain used for inoculation. With strains of apparently equal effectiveness in nitrogen fixation, the extent of excretion may vary considerably, so that actually such strains differ in their effectiveness.

6. The extent of excretion depends also on the quantity of medium available. We have shown that it rises with increasing amounts of sand. We had earlier assumed this as a result of increased air content in the medium. It seems, however, that the air content is decisive in its influence only in respect of the amount of the total fixed nitrogen, but not in regard to the extent of excretion. If, when large quantities of watering solution are employed, the rate of transpiration is high, the extent of excretion shows an apparent fall, since the nitrogen compounds are washed off the sand, and will then be absorbed by the roots of the plant.

7. Nitrate dressing seems to depress the extent of excretion more than it depresses the actual process of nitrogen fixation. Further work is needed, however, to settle this point.

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Fig. 1. Sterilization apparatus for seeds.

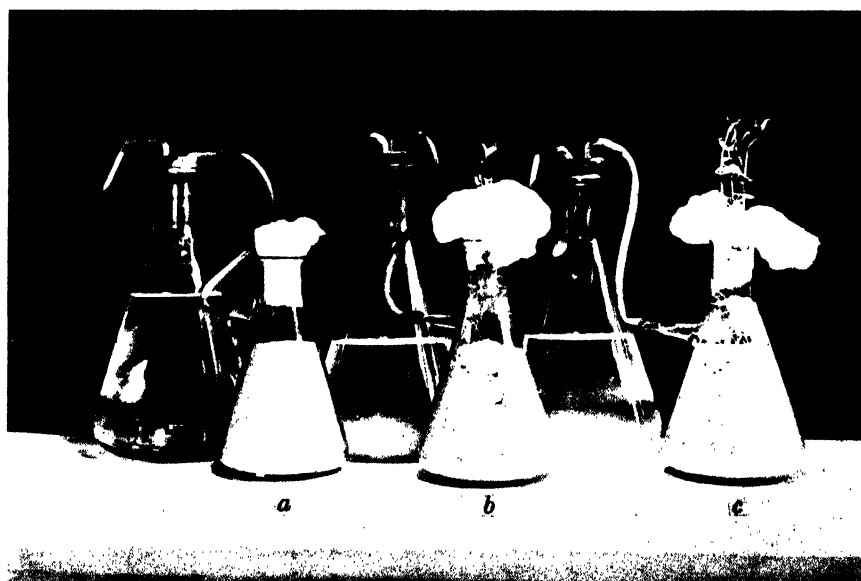


Fig. 2. Sterile plant cultures. *a*, sterile culture flask before planting the test plants; *b*, sterile culture flask with freshly planted test plants; *c*, sterile culture flask after the plants had grown for 1 day.





# INVESTIGATIONS INTO THE ENVIRONMENT AND NUTRITION OF THE CULTIVATED MUSHROOM *PSALLIOTA CAMPESTRIS*

## I. SOME PROPERTIES OF COMPOSTS IN RELATION TO THE GROWTH OF THE MYCELIUM

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(With Plate IV)

### INTRODUCTION

COMPOSTS on which the mushroom mycelium fails to make normal growth are a common cause of poor yields and crop failures. No investigation into the matter appears to have been made, though other aspects of mushroom nutrition have received attention. A good deal of research has been directed towards the discovery of the principal mineral (3, 5, 6, 7, 11) and organic (1, 2, 4, 6, 7, 8, 9) nutrients required by the mushroom. Environmental factors such as temperature (2), partial pressures of gases (12), concentration of soluble materials (6, 7), hydrogen-ion concentration (10, 11) and moisture content (7) of the compost have been studied. But failures occur, of the kind described, when these factors appear to be favourable and when the compost contains ample food material. It has been suggested (1, 2) that poor composts may be unsuitable in physical condition or contain injurious compounds. The influence of the physical or physicochemical condition of the compost on mycelial growth deserves attention, since a compost is a colloidal system composed of solid, liquid and gas phases. In such a system it is possible, without changing the chemical identity of the materials, to bring about great changes in free surface energy, kinetic energy, interfacial tensions, dispersion and hydration of colloids, electrokinetic phenomena, diffusion and osmotic pressures—all of which are probably of vital importance in the nutrition of the mushroom. The properties of stability, swelling and degree of dispersion of most colloidal systems are determined by the nature of the adsorbed ions, especially the adsorbed cations. In the present paper it is shown that, in the case of mushroom composts, the adsorbed cations are of similar fundamental importance, and that by base exchange the

### 352 *Environment and Nutrition of Cultivated Mushroom*

of energy for its uptake, is doubtless of most use to it, and in the experiments has been estimated by subjecting the moist compost to an arbitrary pressure—that between the thumb and fingers. Essentially the same test is used by growers to judge the moisture in composts and, despite its apparent crudity, is quite reliable. Composts were considered to be at similar moisture levels for mycelial growth if they just exuded free water under pressure, and additions of water were made to them until this occurred.

*Sterilization and inoculation.* For the purpose of observing growth rates and the character of the mycelium, the moist compost was pressed firmly into  $6 \times \frac{3}{8}$  in. test-tubes, and the end of the tube plugged with cotton-wool. All samples were steam sterilized for 1 hour at 20 lb. pressure prior to inoculation with spawn. Undoubtedly this process alters the physical nature of the compost, e.g. materials like starch would be dispersed, and certain proteins would be coagulated, and unfortunately it is not possible in a particular case to say with certainty in what manner the changes may affect mycelial growth. Several instances are known to the writer where growth has been improved by sterilization and by increasing the time of sterilization beyond that necessary to kill organisms in the compost; in others, sterilization has apparently impaired the compost. Probably the effect of sterilization on mycelial growth depends on several factors such as the nature of the compost, its previous treatment, and the time of sterilization. After sterilization, the tubes were inoculated with a small piece of pure culture spawn placed, under aseptic conditions, on the top of the compost and pressed against it by means of the cotton-wool plug. The tubes were then put into a ventilated tin box in which a moist atmosphere was ensured by a layer of wet cotton-wool, and the box stood in a room kept at 21° C. (70° F.).

*Manner of describing growths.* The appearance of the mushroom mycelium depends on the type and distribution of the hyphae, which may be thin or thick, close together or widely separated, branched or united into strands according to conditions. In order to compare one mycelium with another, the writer has recorded the strength of growth, the density of the mycelium and the extent of growth; when very noticeable, other characteristics such as branching of the hyphae, strand formation, and vigour of attack on the compost have been mentioned. The strength of growth has been related to the thickness of the hyphae, and qualified as weak, average or strong according as the hyphae are thin, of medium thickness, or thick. The density of the mycelium refers

to the closeness of the hyphae to each other, and has been qualified by the terms sparse, thin, medium and dense.

*Measurement of pH.* In an investigation which will be described in a future publication it was shown that it is essential to use a standard procedure for the determination of the pH of composts. The pH value of compost suspensions increases considerably as the proportion of water is increased, and a ratio of 1 part of air-dry compost to 5 parts of water by weight has been adopted. Titration curves show that composts are strong buffers below pH 7 and only moderate ones between pH 7 and 9. The measurements of pH were made with the quinhydrone electrode. The limitations of this electrode<sup>(13)</sup> are realized, and the pH figures given in this paper are admitted to be probably inaccurate but are considered to be low rather than high.

#### *Extraction of composts with various solvents*

In these experiments, the selective action of a number of solvents was utilized in order to discover the nature of the materials in composts that prevent or modify the growth of mycelium. The solvents were ethyl ether s.g. 0.73, 95 per cent ethyl alcohol, 70 per cent ethyl alcohol, and cold distilled water; they were used in sequence in the order given. Five 10 g. lots of finely ground air-dry compost were weighed out. The first was untreated and the others extracted with ether; 3, 4 and 5 were then extracted with 95 per cent alcohol; next 4 and 5 were extracted with 70 per cent alcohol, and finally 5 was extracted with cold water. Part of each residue was packed into a test-tube, and inoculated with mycelium; each experiment was carried through in duplicate. The details of the extractions were as follows:

*Extraction with ether.* The weighed air-dry compost was tipped into a Soxhlet thimble, dried over calcium chloride in a vacuum desiccator for 24 hours, and then extracted with ether in a Soxhlet apparatus for 8 hours. The residue was freed of ether by warming in an oven at 70° C.

*Extraction with alcohol.* The material was refluxed with 200 ml. boiling alcohol for 3 hours, filtered rapidly on a Buchner funnel (Whatman no. 5, 12.5 cm. paper) and rinsed three times with hot alcohol and air-dried by suction on the funnel.

*Extraction with cold water.* The material was digested with 200 ml. cold distilled water for 24 hours, shaking at intervals, afterwards being thrown on to a Buchner funnel (as above), washed three times with cold water, thoroughly drained and air-dried.

All residues from the ether and alcohol treatments were kept in a

### 354 *Environment and Nutrition of Cultivated Mushroom*

vacuum oven at 90° C. for 2 hours to remove the last traces of these solvents.

The polarity of the solvents increases from ether to water as shown by their dielectric constants: ether=4.3, alcohol (pure)=25, water=81. The general effect of the solvents proceeding from ether to water is to bring about:

- (1) Decreasing solubility of compounds of low polarity.
- (2) Increasing dispersion, solvation and solubility of electrovalent compounds.
- (3) Increasing dispersion, solvation and solubility of hydrophilic colloids.

*Mycelial growth after each extraction.* The solvent actions of ether and 95 per cent alcohol produced very little change in the nature of the mycelial growth obtained on the composts studied, even when a certain amount of growth was obtained on the untreated compost. If anything, ether produced a very slight improvement in the growing properties of composts, and the alcohol invariably removed it. The amount of material extracted by these two solvents is very small. The loss by ether extraction is of the order of 1 per cent, and that by alcohol extraction 2-3 per cent. No test of the effect of the substances in the extracts on mycelial growth was made. It is probable that ether has only a solvent action on composts, removing compounds of low polarity such as fats, fatty and essential oils, phospholipins, waxes and certain glucosides, colouring matters and resins. On the other hand, alcohol has, in addition, a chemical and a physicochemical action on the compost. It dissolves out glucosides, sugars, certain salts, etc.; it also combines with free carboxyl groups forming esters, and it may also denature certain proteins and other colloids, a change which is not reversible on subsequent addition of water. Solvation of certain compounds is almost bound to occur, but such combinations are not likely to survive the treatment in a vacuum oven. The "feel" of the moist composts after these treatments is substantially the same as before, from which it is assumed that the effect produced on their physical properties is small. The slight setback to growth caused by alcohol digestion may be due to an ethyl ester combination detrimental to the mycelium; such esters are likely to be hydrolysed in the 70 per cent alcohol, especially as the compost is alkaline. It is concluded that the materials removed from composts by ether and 95 per cent alcohol are not important in determining whether mycelium will develop in a compost. It is possible that they may be slightly detrimental or slightly beneficial, but this has not been ascertained.

In contrast to ether and 95 per cent alcohol, the other solvents, 70 per cent alcohol and cold water, are able to alter composts so that mycelium will grow readily in them. This, however, does not always happen. Digestion with 70 per cent alcohol may lead to no improvement, and subsequent digestion with cold water may or may not do so. But if growth follows the extraction with 70 per cent alcohol, then extraction with cold water invariably improves it and never impairs it. The compounds dissolved out by 70 per cent alcohol are very similar chemically to those dissolved out by 95 per cent alcohol, but the scope of the solvent is widened by the presence of water. Electrovalent compounds, such as salts, dissolve more readily and hydrophilic colloids tend to pass into solution. From 4 to 7 per cent of material is removed by 70 per cent alcohol. The effect of the cold water is to disperse and hydrate the lyophilic colloids, of which composts are largely composed. Those dispersed to the sol condition are taken out by the water. One can only indicate what the latter may be, since, many types of compounds form sols with water. In a general way sols will be formed by the less complex plant and animal products, by the simple proteins, by simple carbohydrates such as sugars, gums and similar substances, and by products of protein and carbohydrate hydrolysis. Water will also increase the solubility of electrovalent compounds. The cold-water digestion removes from 7 to 9 per cent of organic matter, and from 3 to 5 per cent of ash constituents. The effect of this is to alter the physical nature of the compost, which becomes less sticky and absorbs more water up to the point where water can be pressed out with the fingers. The *pH* of the compost is also increased.

One may argue that the water, and to a less extent the 70 per cent alcohol, have removed substances that are toxic to the mushroom mycelium. The problem then to be solved is the manner in which they exert their toxicity. It appears to the writer that there are three possible ways in which they may do this:

(1) They may be toxic on account of their chemical constitution in the way that substances like vanillin and some of the decomposition products of proteins have been found to be toxic to the higher plants<sup>(14)</sup>.

(2) The electrovalent compounds and others may exert too high an osmotic pressure, an explanation favoured by Styer<sup>(7)</sup> in his experiments.

(3) The degree of dispersion of the organic material may be all-important. The mycelium may be unable to make headway in highly dispersed and hydrated material, perhaps for reasons of surface energy or because of poor aeration in composts containing highly dispersed materials.

## 356 *Environment and Nutrition of Cultivated Mushroom*

In considering these possibilities it must be remembered that mycelium will not grow in some composts even after extraction with the above solvents. Yet one would expect that most of the toxic materials would be removed, and certainly all substances which are capable of exerting high osmotic pressure. The third possibility has this to commend it: as the solvent becomes more watery in nature, i.e. as its power to remove substances that form sols with water increases, there is usually a progressive improvement in the type of mycelial growth. As the more highly dispersed materials are removed so does a compost become more suitable for the mushroom mycelium. This may be all that is required to produce good mycelial growth in some composts, but in others growth may still be hindered by an unfavourable degree of dispersion in the insoluble residual material. The idea that high dispersion of the colloidal constituents of composts, whether sol, gel or straw-like in nature, is detrimental to the growth of mycelium, thus affords a complete explanation of the results of the extraction experiments. Further experiments were therefore commenced with the object of examining the effect on mycelial growth of modifying the degree of dispersion of the colloids in composts. At the same time an attempt was made to find out to what extent lack of growth may be attributed to high osmotic pressure or to the presence of toxic chemicals in the compost.

*Absorbed cations and dispersion.* In order to discover how to bring about changes in dispersion, the flocculating effect of various cations on the sols in a water extract of composts was first investigated. These sols carry a negative charge, and would therefore tend to be flocculated by positive ions. The flocculation values of a number of chlorides and dyes were determined in the following way: a water extract was obtained by digesting 40 g. of air-dry compost with 800 ml. water for 24 hours and shaking the mixture at intervals. It was separated by filtering twice on a Buchner funnel through a Whatman no. 5 filter paper. The chlorides used were 5M NaCl, 5M NH<sub>4</sub>Cl, 3.5M KCl, 2M MgCl<sub>2</sub>, 0.2M CaCl<sub>2</sub>, 0.1M HCl, and the dyes 0.02M auramine, 0.02M malachite green, 0.02M crystal violet and 0.02M Congo red. The electrolyte and sol (or dye and sol) were mixed in test-tubes. 10 ml. of sol were first run into the test-tube and then 10 ml. of electrolyte and water ( $x$  ml. electrolyte +  $(10 - x)$  ml. water) quickly added and mixed in. At the end of 1 hour the tubes were examined for signs of flocculation, and at the end of 3 hours for settling of the flocculated material. The lowest concentrations in millimols per litre of an electrolyte (or dye) to produce flocculation and settling were regarded as the flocculation and settling values for that electrolyte.

The results differed slightly according to the compost used but were of similar order in all cases. Table I gives the flocculation values for the water extract of compost no. 8.

Table I

*pH* of control = 7.18.

Temperature = 20° C.

	NaCl	NH <sub>4</sub> Cl	KCl	MgCl <sub>2</sub>	CaCl <sub>2</sub>	HCl	Aura- mine	Mala- chite green	Crystal violet	Congo red
Flocculation value	>3750	>3750	>2625	>1500	40	11.5	3	2	2	>7
Settling value	—	—	—	—	50	12.5	3.2	2	2	>7

These figures show the extraordinary effectiveness of calcium and hydrogen ions and of the basic dyes in reducing the degree of dispersion from that of a sol occupying 20 ml. down to a flocculent precipitate of small volume, 1–2 ml. Owing to the intense colours of all the dyes, except auramine, evidence of flocculation and settling had to be determined by pouring off the top liquid and noting whether any deposit had collected at the bottom of the tube. The figure for Congo red indicates that the acid dyes are without coagulating effect. The reason for including dyes in the experiment will appear later. The figures for Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup> and Mg<sup>++</sup> are outstanding, and from the fact that salting-out effects were not obtained when the salts were at high concentrations, it was concluded that these cations aid or increase dispersion, and give stable sols by reason of their hydration which probably increases from Mg<sup>++</sup> to Na<sup>+</sup>. In the case of one compost slight turbidity was observed with Mg<sup>++</sup>, the least hydrated ion, when at a concentration of 250 millimols per litre, but settling out never took place. The peptizing action of the cations Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup> and Mg<sup>++</sup> has been shown in an even more convincing way. Calcium ions were added to a water extract in sufficient quantity to produce flocculation, together with varying amounts of one of the above ions. The experiments were carried out in test-tubes to which the reagents were added in the following order: 1 ml. CaCl<sub>2</sub> + *x* ml. NaCl (or NH<sub>4</sub>Cl, etc.) + (9 – *x*) ml. water + 10 ml. water extract; at the end of 1 hour the tubes were examined for signs of flocculation and settling. It was found that each one of the cations, when present in sufficient quantity, was able to offset the flocculating action of calcium and keep the colloids in the water extract in the sol condition. The activities of the cations in this respect may be judged from the values of the following ratios which are those of the concentration (in millimols per litre) of Na<sup>+</sup> (or NH<sub>4</sub><sup>+</sup>, etc.) which just prevented flocculation, to the concentration of Ca<sup>++</sup> (50 millimols per



### 358 *Environment and Nutrition of Cultivated Mushroom*

litre); for one particular extract, the ratios were: Na, 3.5 : 1; Mg, 4 : 1; K, 8.5 : 1;  $\text{NH}_4$ , 10 : 1; and for other extracts the ratios were of a similar order; sodium and magnesium ions appear to be the most active. Higher concentrations of  $\text{Mg}^{++}$ ,  $\text{K}^+$  and  $\text{NH}_4^+$ , however, produce flocculation, an action that does not take place when calcium ions are not present; the ratio of concentration of cation, above which flocculation took place, to concentration of  $\text{Ca}^{++}$  was of the order: Mg, 8 : 1; K, 21 : 1;  $\text{NH}_4$ , 25 : 1; sodium, though tried at a concentration of 2.25 mols per litre (ratio to calcium of 45 : 1), did not bring about flocculation; possibly calcium ions assist "salting-out" of colloidal material by these cations.

The action of cations on the water-soluble material in composts should resemble their action on the other constituents, and by the use of the cations,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$  and  $\text{Na}^+$ , it should be possible to bring a compost to various states of dispersion. This was shown to be the case in the following experiments, in which composts were prepared that were almost saturated with one of the above ions. 10 g. portions of finely ground air-dry compost were extracted with cold water, in the manner adopted in the earlier experiments, until material ceased to pass into solution. They were then digested with 200 ml. of a 0.5 *N* chloride solution and, after 24 hours, thrown on to a Buchner funnel and washed well with distilled water until nearly free of chlorides, thoroughly drained and air-dried. Some general observations on the actions of the various chlorides on composts are set out in Table II below.

Table II

Chloride solution	Ca	Mg	K	$\text{NH}_4$	Na
Rate of settling of compost	Rapid	Moderate > K	Moderate	Slow > Na	Slow
Colour of liquid after 24 hours	Almost colourless	Pale yellow	Deep yellow	Deep yellow	Light brown
Colour of filtrate after removal of salts	Colourless	Yellow	Brown	Light brown	Dark brown
Rate of filtration in drops per min.: compost no. 7	Rapid	136	37	31	30
Greasiness of washed residue	Friable not greasy	Slightly greasy	Greasy	Greasy > K	Greasy > $\text{NH}_4$
Colour of washed residue	Light brown	Brown	Brown	Dark brown	Dark brown
pH of washed residue: compost no. 7	7.8	8.3	8.9	8.5 falling to 7.2	8.9

Colour in the liquids is evidence of material being in solution and, remembering that the composts had been water-washed, it is also an indication that the degree of dispersion of the compost has been increased. Dispersion has therefore been brought about by all the cations except

calcium. The depth of colour is not as readily interpreted, but it is probably true to say that the deeper the colour the more material there is in solution, and the higher the degree of dispersion of the insoluble components of the compost. On this basis the cations are shown to produce different degrees of dispersion which increase from  $Mg^{++}$  to  $Na^+$ . The same conclusion may be drawn from the relative greasiness of the residues, though this property must also depend on the extent of hydration brought about by the respective cations. The rate of settling of the compost and the rapidity of filtration of the liquids are sound indications of the state of aggregation of the finer particles. Calcium is thus shown to be very active in producing aggregation, and the only cation of those studied to do so.

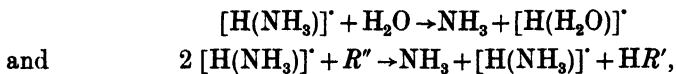
*Adsorbed cations and mycelial growth.* The nature of the mycelium which developed on composts treated in the above manner was compared with that on water-extracted and untreated composts. The strength, density and extent of mycelial growth in a few representative cases 28 days after inoculation are recorded in Table III. The improvement in growth brought about by adsorbed calcium is outstanding and consistent, and is most strikingly illustrated by compost no. 6, which was highly decomposed, black and greasy in its untreated state. The mycelial growth is always strong and dense, and the compost has the appearance of being very vigorously attacked. The effect of calcium can hardly be due to its nutrient qualities, since all the untreated composts contained some calcium, and yet on most of them no trace of mycelium would develop. The other ions, especially sodium, potassium and magnesium, invariably cause a falling off in growth compared with the growth on water-extracted material. From the fact that these ions do, in some cases, allow the growth of a strong mycelium as, for example, on compost no. 9, it seems that none of them is directly toxic to the mycelium. It is reasonable to conclude, therefore, from these experiments that the mushroom mycelium needs a medium which is not highly dispersed, and that the correct degree of dispersion is the one given by an excess of calcium ions. The ease of dispersion of a compost is clearly a property that depends on the nature of its constituents, some of which are more readily dispersed than others. The effects of dispersing agents on different composts will therefore vary in degree, and in this manner the irregular effects of sodium, potassium and magnesium ions may possibly be explained.

The results for ammonium appear to be anomalous since the dispersion caused by this ion is almost as extensive as that caused by sodium. But

Table III. *Effect of adsorbed bases on mycelial growth*

No. of compost	Untreated	Water extracted	With adsorbed Na	With adsorbed NH <sub>4</sub>	With adsorbed K	With adsorbed Mg	With adsorbed Ca
9	Weak, thin, 2 cm. pH 7.9	Strong, medium, strandy, 10 cm. 8.9	Strong, medium, strandy, 9 cm. 9.0	Strong, dense, 7.5 cm. 8.6 falling to 7.5	Strong, medium, strandy, 9 cm. 9.0	Strong, medium, strandy, 9 cm. 8.3	Strong, dei 9 cm. 8.1
7	Weak, medium, 5.5 cm.	Strong, medium, strandy, 9.5 cm.	Average, medium, strandy, 10 cm.	Strong, dense, strandy, 10 cm.	Average, medium, strandy, 10 cm.	Weak, medium, strandy, 8 cm.	Strong, dei 10 cm.
1	pH 8.4 No growth	8.8 Average, dense, 10 cm.	8.9 Weak, thin, 1 cm.	8.5 falling to 7.2 Average, dense, 7 cm.	8.9 Weak, thin, 2 cm.	8.3 Weak, thin, 8 cm.	7.8 Strong, dei 8 cm.
3	pH 7.5 No growth	8.5 Average, medium, 9.5 cm.	8.6 Weak, thin, 7 cm.	8.2 falling to 7.3 —	8.4 Weak, thin, 6.5 cm.	7.7 Weak, thin, 9.5 cm.	7.5 Strong, dei 10 cm.
6	pH 7.8 No growth	7.8 No growth	8.3 No growth	— Weak, thin, 3 cm.	8.3 No growth	7.6 No growth	7.3 Strong, dei 10 cm.
	pH 7.6	8.5	8.8	8.3 falling to 7.2	8.7	8.1	7.9

it was noticed that, whilst the ammonium composts were air-drying, they fell a whole unit in  $pH$  in 60 hours. In other words there was a tenfold increase in  $pH$ . This could be brought about by dissociation of the ammonium ion and its substitution by hydrogen ions according to such reactions as the following:



where  $R''$  represents part of the surface of the adsorbing complex; or perhaps by conversion of ammonia into nitric acid. With the fall in  $pH$ , the compost appeared to lose some of its greasiness, as would be expected from the known action of hydrogen ions in causing aggregation of the particles in composts. The use of ammonium ions as a source of nitrogen by the mycelium will similarly make the compost more acid and less greasy. Very probably the dispersed condition of a compost caused by ammonium ions is transitory under the conditions of the experiment, and this, in conjunction with the nutrient value of the ion to the mycelium, explains the slight improvements produced in mycelial growth.

The effect of  $pH$  on the development of mycelium will be discussed in a later section. Here it may be noted that the  $pH$  of a compost is altered by the adsorption of cations (other than hydrion). This is to be expected as the titration curves show that composts are not strongly buffered in the region  $pH$  7-9. Each cation produced the same order of  $pH$  value, in relation to that produced by the other cations, in all of the composts which were studied. Sodium and potassium gave the highest  $pH$  values, ammonium a slightly lower value, magnesium lower still, and calcium the lowest  $pH$  value.

*Dispersion and mycelial growth.* The influence of the degree of dispersion of composts on mycelial growth has been further studied, using sodium ions to increase the dispersion and calcium ions to reduce it. The experiments with composts nearly saturated with a single cation and, more particularly, the experiments on the flocculation of water extracts, show that high hydration, dispersion and solubility of a compost should be brought about by the removal of calcium and hydrogen ions from the compost, and their substitution by sodium ions in the presence of anions such as  $OH'$  and  $CO_3''$ . Improved mycelial growth has been obtained on composts from which highly dispersed material has been removed in the sol condition, and the residue then flocculated with calcium ions. It would be of great practical value to know whether it is necessary to remove soluble material and to what extent this should be

### 362 *Environment and Nutrition of Cultivated Mushroom*

carried, or whether flocculation of the entire compost, water-soluble material included, with calcium ions is all that is needed to obtain good mycelial growth. The following experiments were designed to provide this information. The effect on mycelial growth was determined of:

(a) Increasing the dispersion and the extraction of material from composts by means of the solvents, cold water,  $N/20$   $\text{Na}_2\text{CO}_3$  and  $N/20$   $\text{NaOH}$ .

(b) Increasing the extraction of material by the methods adopted in (a), but afterwards reducing the dispersion of the residue by means of  $N/20$   $\text{CaCl}_2$  solution.

(c) Reducing both the dispersion and the extraction of material from composts by (i) digestion with  $N/20$   $\text{CaCl}_2$  only, and (ii) avoiding extraction of material by mixing powdered calcium sulphate into the compost.

Nine treatments were thus given to each compost; they were:

- A. No treatment.
- B. Extraction with cold water.
- C. B followed by digestion with  $N/20$   $\text{CaCl}_2$ .
- D. Extraction with  $N/20$   $\text{NaOH}$ .
- E. D followed by digestion with  $N/20$   $\text{CaCl}_2$ .
- F. Extraction with  $N/20$   $\text{Na}_2\text{CO}_3$ .
- G. F followed by digestion with  $N/20$   $\text{CaCl}_2$ .
- H. Extraction with  $N/20$   $\text{CaCl}_2$ .
- J. Addition of 1 per cent of  $\text{CaSO}_4$ .

The weight of calcium sulphate used is equivalent in calcium content to three-quarters of the weight of calcium chloride brought into contact with the composts. Each treatment was carried through in duplicate on 10 g. of finely ground air-dry compost. The compost was digested for 24 hours with 200 ml. of the appropriate liquid—water,  $N/20$   $\text{NaOH}$ ,  $\text{Na}_2\text{CO}_3$  or  $\text{CaCl}_2$  solutions—filtered on a Buchner funnel and washed with cold distilled water till the filtrate became colourless. The residues requiring treatment with calcium chloride solution were returned to the flask and digested for 4 hours with  $N/20$   $\text{CaCl}_2$ , then filtered and washed with water till almost free of chlorides. The  $\text{NaOH}$  and  $\text{Na}_2\text{CO}_3$  extracts were almost black in colour and generally much darker than the water extract. Of the  $\text{CaCl}_2$  extracts, H was light brown in colour, C was very faintly yellow and E and G were colourless. As in previous experiments depth of colour was regarded as an indication of the amount of material in solution; treatments D, E, F and G, therefore, removed the greatest

amounts of material from the composts, B and C somewhat less, H very little and J none at all. The residues from the NaOH and  $\text{Na}_2\text{CO}_3$  treatments, D and F, were extremely greasy, D more so than F, whilst that from water extraction, B, was often on the greasy side, but the residues from all the  $\text{CaCl}_2$  treatments were non-greasy and friable. There were obvious differences, however, in the  $\text{CaCl}_2$  residues; E and G settled out rapidly to leave a clear liquid, C and H relatively slowly; filtration was similarly fast with E and G and relatively slow with C and H; perhaps these differences were due to the removal of more of the fine particles from the composts by the dispersing actions of the NaOH and  $\text{Na}_2\text{CO}_3$  solutions.

Table IV. *Effect of dispersion on mycelial growth*

No. of compost	Treatments				
	A	B	C	D	E
9	Weak, thin, 3 cm. pH 7.9	Strong, medium, strandy, 10 cm. 8.8	Strong, dense, 10 cm. 8.0	Weak, thin, 0.5 cm. 9.7	Strong, dense, 9 cm. 8.7
7	Weak, medium, 5.5 cm. pH 8.4	Strong, medium, strandy, 8 cm. 8.8	Strong, dense, 9 cm. 8.0	Weak, medium, strandy, 6 cm. 9.4	Strong, medium, 10 cm. 8.5
8	No growth pH 7.5	Weak, medium, 10 cm. 8.6	Average, dense, 10 cm. 7.7	No growth 9.4	No growth 9.5
6	No growth pH 7.6	No growth 8.5	Strong, dense, 10 cm. 7.9	No growth 9.5	Strong, medium, 9 cm. 8.6
1	No growth pH 7.5	Average, dense, 9 cm. 8.5	Strong, dense, 10 cm. 8.0	No growth 9.3	Average, medium, 8 cm. 8.6

No. of compost	Treatments			
	F	G	H	J
9	Average, medium, strandy, 8 cm. pH 9.4	Strong, dense, 9 cm. 8.5	Strong, dense, 10 cm. 7.5	Strong, dense, 7.5 cm. 7.7
7	Average, medium, strandy, 7.5 cm. pH 9.0	Strong, dense, 10 cm. 8.2	Strong, dense, 9 cm. 8.0	Strong, dense, strandy, 9 cm. 7.9
8	No growth pH 9.1	Average, dense, 9.5 cm. 8.9	Average, dense, 10 cm. 7.3	Average, dense, 8 cm. 7.3
6	No growth pH 9.2	Strong, medium, 8.5 cm. 8.4	Strong, dense, 10 cm. 8.0	Strong, dense, 6 cm. 7.5
1	No growth pH 9.0	Average, dense, 8.5 cm. 8.3	Strong, dense, 10 cm. 7.6	Strong, dense, strandy, 5 cm. 7.3

### 364 *Environment and Nutrition of Cultivated Mushroom*

The strength, density and extent of mycelial growth on the above residues, 28 days after inoculation, are recorded in Table IV for a few of the composts which were studied.

The treatments D and F give a high pH value to composts, and mycelial growth is probably affected as much by this factor as by any other. The results for treatments B, C, E, G and H, however, show that there appears to be no advantage at all in removing colloidal material, provided this is effectively flocculated by calcium ions. But the results for treatment J, in which the purpose was flocculation without the removal of any material whatsoever, are not as good as those given by treatment H. It may be that calcium sulphate, despite its low solubility, increases the osmotic pressure unduly; the salt was chosen to avoid this as much as possible, and yet provide sufficient calcium ions for flocculation. It is possible that flocculation was not efficient; it was noticed that composts treated with calcium sulphate were greasy as compared with composts digested with calcium chloride solution; the calcium sulphate was mixed into the air-dry compost as thoroughly as possible and the mixture then moistened, but calcium sulphate is not very soluble, and contact with the compost is obviously not as thorough as by suspending the compost in a solution of a highly soluble calcium salt. This explanation seems plausible for two reasons: (1) increasing the amount of calcium sulphate from 1 to 1.5 per cent gives better results, and (2) extracting composts with saturated calcium sulphate solution is as effective as extraction with calcium chloride solution. On the other hand, digestion with calcium chloride solution may be a superior treatment because water-soluble substances detrimental to the mushroom mycelium and not precipitated by calcium ions are removed; the effect of water extracts on the growth of mycelium has, therefore, been investigated as described in the following section.

*Water-soluble substances and nutrition.* According to Duggar<sup>(2)</sup>, *Psalliota campestris* does not grow readily in liquid media, and nutrient solutions are best examined by absorbing them on to a porous solid. The probable reason for poor growth in aqueous solutions is that the mushroom mycelium is strongly aerobic, and the aeration in liquids is inadequate for its needs<sup>(7)</sup>. It is therefore not possible to test out the effect of the water-soluble substances in composts on mycelial growth by direct inoculation of a water extract with a fragment of spawn. A porous solid is required on which to absorb the extract, and it should be chemically pure, of uniform quality, suitable as an environment for the mycelium but not attacked by it, and should take up the materials in

a water extract without changing them; unfortunately it is not possible to obtain a material with all these qualities. Duggar<sup>(2)</sup> used commercial grey filter paper; Styer used a purer paper which was dyed black to show up mycelial growth<sup>(6)</sup>, and he also used a silica gel<sup>(7)</sup>, but none of these materials, though in many ways suitable, is inert chemically, for all of them have reactive surfaces at which ions and colloids may be adsorbed; they appear, however, to be the best available, and filter paper has been used in the present case.

Two series of experiments were carried out; in one, water extracts were absorbed on to filter paper and the product inoculated with spawn; in the other, mineral salts were also added with the idea of guarding against a mineral deficiency which would prevent growth and obscure the nutrient qualities of the water extracts.

*Preparation of the filter paper.* In order to make the mycelial growth easily visible, a suitable background was made for it by dyeing the filter paper. The experiments on the flocculation of water extracts of composts show the need for discrimination in the choice of dye; basic dyes flocculate the soluble material very readily; acid dyes do not cause flocculation; in experiments in which the properties of a water extract are being investigated, basic dyes should, therefore, not be used. A direct cotton dye of the acid type, Congo red, was chosen, though a direct cotton black might be better; Congo red, however, was available and its constitution was known and in practice was found to be very suitable. Congo red is derived from benzidine and contains nitrogen in azo and amino groups, a fact which must be borne in mind. The method of dyeing was as follows: 4 g. Congo red, 200 g. NaCl and 1 litre distilled water were heated to boiling, the flame removed, and 50 g. filter-paper clippings (Whatman's ordinary) added; the mixture was covered with a clock-glass and stirred occasionally. At the end of 8 hours the dyed paper (pulp) was poured on to a Buchner funnel, washed under the tap until all the excess dye was removed, and drained by applying pressure and suction; the paper was then broken up, brought to air-dryness and disintegrated in a Christie and Norris high-speed mill from which the screen had been removed. The product was uniform in texture and colour, and bulky; it contained nitrogen in the dye and probably sodium and calcium acquired during the dyeing process.

*Preparation of the water extracts.* Cold-water extracts of composts were obtained by shaking 50 g. finely ground air-dry compost with 500 ml. cold distilled water for 24 hours and filtering on a Buchner funnel through a Whatman no. 5 paper.



## 366 *Environment and Nutrition of Cultivated Mushroom*

*Composition of the nutrient mineral solutions.* The nutrient mineral solutions were those used by Styer<sup>(6)</sup>. Solution A consisted of:  $\text{NH}_4\text{NO}_3$ , 0.1 M;  $\text{KH}_2\text{PO}_4$ , 0.04 M;  $\text{MgSO}_4$ , 0.02 M;  $\text{K}_2\text{SO}_4$ , 0.01 M;  $\text{CaCl}_2$ , 0.002 M;  $\text{FeSO}_4$ , a trace; and sufficient NaOH to bring the pH to 6.0. Solution B was similar but did not contain  $\text{NH}_4\text{NO}_3$ .

*Method of absorbing the extracts on paper.* Each test was carried out in  $6 \times \frac{3}{8}$  in. test-tubes, and for this size tube 4 g. filter paper was found to be a suitable quantity; the tests were in duplicate. On 8 g. paper the water extract from 8 g. compost was absorbed, i.e. 8/50 of the volume of water extract obtained as described above; the extract was absorbed 22–24 ml. at a time, air-drying after each addition, or in such quantities that 18 ml. was left over for bringing the paper to a suitable moisture content for mycelial growth (this gives a ratio of air-dry paper to water of 225/100, which is within the favourable range determined by Styer<sup>(7)</sup>, and appears to be correct as judged by pressing between the thumb and fingers). When mineral salts were also included, the paper was brought to air-dryness after all the water extract had been added, and then moistened with 18 ml. solutions A or B. After filling, each tube was plugged with cotton-wool, sterilized, inoculated with a fragment of pure culture spawn and cared for as already described. The strength, density and extent of mycelial growth, 28 days after inoculation, are recorded for seven different extracts in Table V.

Growth was obtained on all the water extracts, but the rate of growth was slow; the quality of the mycelium was never poor, and in two instances ((3), (6)), was very good. This is in striking contrast to the mycelial growth on the corresponding composts; on five of the latter ((3), (6), (8), (1), (4)), there was no growth at all and on two ((7), (9)), the growth was poor; very good results were obtained on the water extracts of two of the worst composts ((3), (6)). On the corresponding water-washed composts, growth was improved as a result of the removal of the water-soluble materials in all cases except (6), which was again a failure; very good growth was obtained, however, on the water extract from (6). In three cases ((3), (6), (8)), the quality of the mycelium was better on the water extract than on the water-washed compost, and in four cases ((1), (4), (7), (9)), it was poorer. Rate of growth was definitely improved by extracting the composts with water, the rate sometimes being two and three times faster than on the water extracts.

It seems to be quite clear from these results that there are no toxic compounds in the water extracts; in fact, the extracts have marked nutritive value; but the rate of growth on the extracts is slow as if

Table V. *Effect of water extracts of composts on mycelial growth*

No. of compost Treatment	(3)	(6)	(8)	(1)	(4)	(7)	(9)
Extract + paper	Strong, dense, 5.5 cm. pH of extract 7.9 pH on paper 7.4	Strong, dense, 6 cm. 7.9 7.4	Average, medium, 4.5 cm. 7.8 7.3	Average, medium, 3 cm. 7.9 7.5	Average, medium, 5.5 cm. 8.0 7.4	Average, medium, 5.5 cm. 8.2 7.5	Average, medium, 5 cm. 7.9 7.1
Extract + paper + solution B	Average, thin, 5 cm.	Average, thin, 4.5 cm.	Average, dense, 10.5 cm. pH 6.8	No growth	Average, medium, 6 cm.	—	Weak, thin, 1 cm.
Extract + paper + solution A	Average, medium, 6 cm.	Average, medium, 3 cm.	Average, dense, 7 cm. pH 6.3	No growth	Average, medium, 5 cm.	Average, medium, 7 cm.	Weak, thin, 4 cm.
Untreated compost	No growth pH 7.8	No growth 7.6	No growth 7.5	No growth	No growth	Weak, medium, 5.5 cm.	Weak, thin, 3 cm. 7.9
Water- washed compost	Average, medium, 9.5 cm. pH 7.8	No growth 8.5	Weak, medium, 10 cm. 8.6	Average, dense, 9 cm. 8.5	Strong, medium, 9 cm. 8.4	Strong, medium, 8 cm. 8.8	Strong, medium, 10 cm. 8.8

### 368 *Environment and Nutrition of Cultivated Mushroom*

progress were hindered for physical reasons. The same is true of the water-extracted composts, except that the physical state appears to be more favourable and the rate of growth correspondingly good. It appears also that the quality of the mycelium is not adversely affected by the water-soluble substances in composts when the latter are absorbed on filter paper, but the water-soluble substances are definitely detrimental in association with the insoluble constituents of composts. The writer is of the opinion that the explanation of these facts lies in the tolerance of the mushroom mycelium to material which is highly dispersed, and if the latter is present in sufficient quantity then growth of the mycelium is hindered or stopped; the degree of dispersion of the insoluble material is contributory and is responsible for the better growth on filter paper plus water extract compared with the growth on the untreated composts. The results for water extracts plus minerals (without ammonium) support this idea; the minerals consist almost entirely of salts which increase the dispersion of the materials in water extracts, and on the whole the mycelial growths were poorer when they were included; in one case only, (8), were the rate of growth and quality of the mycelium better, and this may well have been due to the nutritive value of the salts. The inclusion (with the salts) of ammonium nitrate, the cation of which aids dispersion, gave similar results; the quality of the mycelium was on the whole improved, probably on account of the nutrient properties of ammonium nitrate, but the peptizing action of the ammonium ion was apparent in reducing rate of growth.

Other experiments with water extracts absorbed on filter paper also indicate the significance of dispersion; in these experiments the water extract absorbed on the paper was reduced to three-quarters and one-quarter the full quantity, and to none at all; two series were run, one with water extract and solution A, and another with water extract and solution B. The strength, density and extent of mycelial growth, 28 days after inoculation of the extracts (from the composts used in the preceding experiments), are recorded in Table VI.

The experiments bring out several interesting facts. In the first place, a medium made up of filter paper and the mineral salts in solution B is of very low nutritive value to the mushroom mycelium; very few hyphae grow into it from the spawn, and they are mostly extremely thin and difficult to see with the unaided eye; probably most of the energy for growth is derived from the fragment of spawn and hardly any from the paper (cellulose). Somewhat better growth is obtained when ammonium nitrate is also present, but growth is still poor; the mycelium

Table VI. *Effect of amount of soluble material in composites on mycelial growth*

No. of compost ... Treatment	(3)	(6)	(8)	(1)	(4)	(7)	(9)
Full water extract and solution B on paper	Average, thin, 5 cm.	Average, thin, 4.5 cm.	Average, dense, 10.5 cm. pH 6.8	No growth	Average, medium, 6 cm. 6.5	—	Weak, thin, 1 cm. —
$\frac{1}{2}$ water extract and solution B on paper	Weak, thin, 4.5 cm.	Average, dense, 10.5 cm.	Average, moderate, 10.5 cm. pH 6.7	Average, medium, 10 cm. 6.6	Average, medium, 7 cm. 6.4	—	Average, medium, 7 cm. —
$\frac{1}{4}$ water extract and solution B on paper	Weak, thin, 8.5 cm.	Strandy, thin, 10.5 cm.	Average, thin, 10 cm. pH 6.5	Average, thin, 10.5 cm. 6.3	Average, dense, 9 cm. 6.3	—	Average, dense, 11.5 cm. —
No water extract; solution B on paper	Weak, sparse, 4 cm.	Weak, sparse, 5 cm.	Strandy, sparse, 4.5 cm.	Weak, sparse, 6 cm. pH 6.0	Weak, sparse, 4.5 cm. 6.0	—	Weak, sparse, 6 cm. —
Full water extract and solution A on paper	Average, medium, 6 cm.	Average, medium, 3 cm.	Average, dense, 7 cm. pH 6.3	No growth	Average, medium, 5 cm. 6.4	Average, medium, 7 cm.	Weak, thin, 4 cm. —
$\frac{1}{2}$ water extract and solution A on paper	Average, dense, 9 cm.	Average, dense, 6.5 cm.	Average, dense, 7.5 cm. pH 6.2	Average, medium, 8 cm. 6.2	Average, medium, 5 cm. 6.3	—	Average, medium, 5 cm. —
$\frac{1}{4}$ water extract and solution A on paper	Average, medium, 11.5 cm.	Average, medium, 9.5 cm.	Average, medium, 10 cm. pH 6.2	Average, medium, 11 cm. 6.0	Average, dense, 8 cm. 5.9	Average, medium, 10 cm.	Average, medium, 9 cm. —
No water extract; solution A on paper	Weak, thin, 11.5 cm.	Weak, thin, 10.5 cm.	Strandy, thin, 9.5 cm. pH 6.0	Strandy, thin, 11 cm.	Weak, thin, 10 cm.	Weak, thin, 9.5 cm.	Weak, thin, 10 cm. —

### 370 *Environment and Nutrition of Cultivated Mushroom*

is more extensive and not quite so thin, and the hyphae are a little stronger; the ammonium nitrate is probably of direct nutritive value, and may also help the mycelium to obtain energy from the paper; results similar to this were obtained by Styer<sup>(6)</sup>.

The addition of water extract to the paper and mineral salts makes a very big difference to the rate at which the hyphae grow, to their thickness and to their closeness together; the paper also appears to be attacked. With one-quarter the full amount of water extract and the salts in solution A, a well-developed, rapidly growing mycelium is obtained; on increasing the quantity of water extract to three-quarters the full amount, the rate of growth is reduced, but the density of growth is very often improved; with the full quantity of water extract the rate of growth is still further decreased and the quality of the mycelium also suffers.

It seems that the water extracts of these composts contain materials which in small amount promote a strong and rapid growth of mycelium, but when they are in excess of a certain quantity there is a marked reduction in the strength and rate of growth. An exception to this is provided by compost no. 8; the water extract of this compost gave better results as it was increased in amount, yet no growth was obtained on the compost itself, and only a very poor one after it had been water-washed; however, growth was excellent after flocculation of the compost with calcium ions. This seems to be a clear case of growth being prevented through the insoluble constituents of the compost being too highly dispersed. In the other cases, the amount of water-soluble material, as well as the dispersion of the insoluble material, is of importance. The simplest explanation, supported by the experimental work, of the action of water-soluble substances from composts of reducing mycelial growth, appears to be that the mushroom mycelium is unable to tolerate more than a certain amount of material in the sol condition. It is possible, as the work of Styer<sup>(6, 7)</sup> suggests, that the osmotic pressure of the soluble materials is also a factor which limits growth, since the addition of soluble salts to paper plus water extracts reduces mycelial growth (see Table V), but the effect may equally be attributed to the dispersing action of the cations of the salts on the paper and absorbed water extract as a whole. In any case, the sols in composts and in water extracts of composts are not likely to exert more than small osmotic pressures, and when they are flocculated good mycelial growth is invariably obtained, so that in composts the effect of osmotic pressure does not appear to be important; the point can only be decided, however, by

actual measurements of osmotic pressure in the composts themselves. The effect of ammonium nitrate is similar in these experiments to that observed when using the full amount of water extract; as a general rule it repressed the rate of growth but improved the density of the mycelium, which suggests that it has nutritional value but is detrimental in other ways, e.g. by increasing the degree of dispersion or the osmotic pressure.

### DISCUSSION

The experimental work has shown that the development of the mushroom mycelium is intimately connected with the degree of dispersion of the various constituents of the compost. In highly dispersed material the mycelium grows only slowly or not at all, but when the dispersion is reduced by flocculation with calcium ions, the mycelium will grow readily and vigorously. The flocculation must be of a specific kind; it is not sufficient to flocculate sols to the condition of gels and to reduce the dispersion of the insoluble phases, but the coagula and other constituents must be devoid of sliminess and greasiness; if a compost is at all greasy, mycelial growth is checked. Flocculation must result in a compost which is non-greasy, friable and granular, and these properties are conferred on composts by coagulating them with calcium ions. The reason why such a physicochemical change as flocculation should make a compost more suitable for the nutrition and growth of the mushroom mycelium is not known. Flocculation coincides with a reduction in surface area and a redistribution of free energy, especially surface energy; since the surface area is reduced one might expect the surface energy to be reduced, but this is not so, for after a compost has been flocculated with calcium ions, more water must be added to it to reach the point when free water can be squeezed out between the thumb and fingers—more water is held against the same applied force, in other words, the surface energy and the interfacial tensions are greater. Flocculation also brings about changes in the structure of surfaces, and in electrokinetic phenomena. It is possible that these changes in surface energy, surface structure and potential promote enzymatic actions which are favourable to the mushroom mycelium, or assist diffusion across interfaces of food materials and excretory products. Another improvement in composts brought about by flocculation is that aeration is better, but one cannot regard this as being entirely responsible for better mycelial growth, although it goes hand in hand with it, since on really bad composts not even a surface mycelium will develop.

Although it is not possible to offer an explanation, other than of mere

## 372 *Environment and Nutrition of Cultivated Mushroom*

speculation, of the experimental results, it is possible to derive information of practical value and for planning future experimental work. The fact that the cations Na,  $\text{NH}_4$ , K and Mg tend to promote dispersion suggests that the mineral composition of feeding stuffs and bedding of the animals may exert an influence on the greasiness or otherwise of composts, especially as growers recognize the kind of ration given to the horse to be important. In the experiments on flocculating water extracts of composts, it was shown that for a particular concentration of calcium ions (100 milli-equivalents per litre) quite a small excess of the above cations would prevent flocculation, the actual ratios to calcium in equivalents being: Na, 1.6 : 1;  $\text{NH}_4$ , 5 : 1; K, 4.3 : 1; Mg, 4 : 1. It is not suggested that these figures are invariable and apply to all composts and all quantities of calcium, but they serve as a guide in considering the relation of the mineral composition of feeding stuffs and bedding materials to the physical state of composts; for this purpose Table VII has been constructed.

Table VII. *Mineral elements in foodstuffs and straws*

Plant product	Ash % of plant product	Cations in mg. equivalents per 100 g. plant product				Ratio of other cations to Ca				
		Na	K	Mg	Ca	Na	K	Mg	Na + K + Mg	
						Ca	Ca	Ca	Ca	
Oats, grain (2)	3.4	7.0	10.8	10.0	5.0	1.4	2.2	2.0	5.6	
Beans, seed (1, 3)	3.2	2.2	27.6	16.0	5.7	0.4	4.8	2.8	8.0	
Beans, seed (2)	3.8	3.5	31.0	15.0	10.5	0.3	3.0	1.4	4.7	
Maize, grain (1)	1.3	1.3	8.5	9.0	0.4	3.2	21.2	22.6	47.0	
Wheat, bran (1)	5.8	8.1	34.0	37.5	4.3	1.9	7.9	8.7	18.6	
Carrots (3)	0.7	3.2	7.9	1.0	1.8	1.8	4.4	0.6	6.7	
Average pasture (1, 4)	2.0	1.6	13.6	3.5	7.1	0.2	1.9	0.5	2.6	
Meadow hay (4)	6.6	9.4	34.0	10.0	26.1	0.4	1.3	0.4	2.0	
Clover hay (1)	7.0	3.9	34.0	22.5	57.1	0.1	0.6	0.4	1.1	
Lucerne hay (2)	6.3	19.6	19.5	30.0	51.5	0.4	0.4	0.6	1.4	
Timothy hay (2)	3.1	13.5	13.8	8.3	8.5	1.6	1.6	1.0	4.2	
Wheat, straw (1)	5.3	9.7	16.0	5.0	12.5	0.8	1.3	0.4	2.5	
Oat straw (1, 3)	4.9	8.1	31.9	5.0	12.5	0.6	2.6	0.4	3.6	
Barley straw (1, 3)	4.6	7.7	25.6	7.5	17.9	0.4	1.4	0.4	2.2	

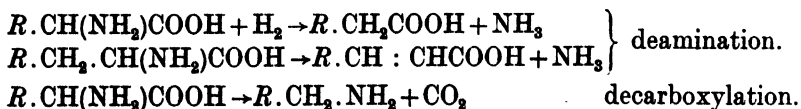
The above are average figures calculated from data in the following publications:

- (1) *Bulletin 156b*, University of Leeds, 1936.
- (2) Armsby, *The Nutrition of Farm Animals*, 1928.
- (3) Wolff, *Aschen-Analysen*, 1871.
- (4) Orr, *Minerals in Pastures*, 1929.

The ratios given in Table VII show that, provided the cations are entirely available for affecting dispersion, i.e. in composts they are entirely exchangeable by other bases, the straws, pasture grass and the hays, with the possible exception of timothy hay, are unlikely to give greasy composts on account of their mineral composition; the same may

possibly be said of oats and beans; but wheat bran, maize and carrots appear likely to do so. Of course the relative proportions of the cations are modified in passing through the horse; some of them are digested and later excreted in different ways; sodium and potassium largely in the urine, calcium and magnesium usually in the faeces. There is, therefore, some uncertainty in arguing from data on the mineral composition of foodstuffs; it would be sounder to carry out mineral analyses of composts and relate these to the physical properties of composts and to mycelial growth. But the figures in Table VII do suggest that certain foodstuffs are better than others from the point of view of the mushroom grower; one may also say that a manure made up from droppings and bedding soaked with urine is inferior for composting purposes to one containing little or no urine.

In addition to the minerals in composts, there is also the ammonium ion to be considered in connexion with dispersion and poor mycelial growth. Ammonia is produced during composting by both aerobic and anaerobic processes, and very bad composts frequently have an ammoniacal smell. In such cases ammonia may be detrimental on account of toxic properties or because ammonium ions cause dispersion; the toxicity of ammonia to mushroom mycelium has not been determined, but in any case the gas cannot be entirely responsible for lack of mycelial growth, since removing ammonia does not turn a bad compost into a good one; greasiness invariably accompanies the smell of ammonia, so that dispersion is indicated as the cause of the trouble, and one must consider whether this is brought about entirely by ammonium ions. It occurs to the writer that there is another possibility if it be assumed that the nitrogen bases, particularly the substituted ammonias or amines, form ions which are as active as the ammonium ion in producing dispersion; such bases are frequently much stronger than ammonium which makes the assumption feasible. Under anaerobic conditions, both ammonia and amines may be produced from amino acids, the former by deamination and the latter by decarboxylation of amino acids:



Decarboxylation takes place under anaerobic conditions only, but deamination occurs also under aerobic conditions. Deamination is not likely to be harmful to the physical condition of composts, in fact since it produces an increase in acidity, it should be of benefit. On the other



### 374 *Environment and Nutrition of Cultivated Mushroom*

hand, decarboxylation destroys an acid and replaces it with an amine which is far less volatile than ammonia and possibly more basic and able to cause extensive dispersion of the constituents of composts. If this be true, it is a sound reason for maintaining aerobic conditions as much as possible during composting; in practice it is noticeable that composts which are overwatered and turned at lengthy intervals tend to become greasy.

Thus there appear to be many agencies which work to bring composts into an undesirable greasy condition, but fortunately the experimental work has brought out the important fact that greasiness may be removed by the adsorption of calcium ions. Since calcium salts are plentiful and relatively cheap, the obvious remedy and security to the grower against composts in bad condition is to add a sufficient quantity of calcium to the manure before composting is begun. We<sup>1</sup> have carried out many experiments and determined that if ground gypsum is added so as to ensure that there will be from 1.5 to 2 per cent of it in the finished compost, then greasiness is prevented and normal growth obtained. An account of the practical procedure has already been published (15, 16), and details of the experiments will appear shortly.

Experiments have been carried out in the past by several workers, to determine the effect of the common fertilizers—potassic, nitrogenous and phosphatic—on mushroom yields. The results have been erratic and indecisive; in some cases the yield was increased slightly and in others reduced. Since many of the constituents of these fertilizers promote dispersion in composts, it is suggested that much more positive results would be obtained if calcium were also added to prevent dispersion; in this connexion the observation of Duggar<sup>(2)</sup> is of interest that, in his experiments, some slight advantage resulted from the use of calcium compounds; the increase in yield obtained by Guffroy<sup>(5)</sup> from the use of basic slag may also have been partly due to flocculation of the compost by the calcium of the slag.

In the experimental work, the *pH* of each compost was measured and the data on *pH* and mycelial growth are of interest. Good, strong mycelial growth was obtained on composts over the range *pH* 6–9 (in one case *pH* 9.4 was measured). These figures are at variance with those of Frear *et al.* (10), who found that growth was best over the range *pH* 6–6.7 and fell off markedly above *pH* 6.7. These workers used a medium of filter paper, casein and mineral salts brought to different *pH* levels by the addition of either sulphuric acid or potassium hydroxide; the mineral

<sup>1</sup> Pizer, N. H. & Thompson, A. J. Experiments 1936, as yet unpublished.

salts were provided by Styer's solution B (p. 366). The particular protein used would be readily dispersed above  $pH$  6.7 by the salts in solution B and by the addition of potassium hydroxide; hence it is suggested that the factor limiting growth in these experiments was not  $pH$  but high dispersion of the casein, and if another medium had been used, different conclusions would probably have been drawn. Bechmann's<sup>(11)</sup> results for beer wort-agar may be criticized similarly.

#### ACKNOWLEDGEMENT

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#### EXPLANATION OF PLATE IV

Fig. 1. Shows the effect on mycelial growth of extracting compost no. 8 with various solvents as described on p. 353. A. Untreated compost. B. Extracted with ethyl ether. C. B, followed by extraction with hot 95 per cent ethyl alcohol. D. C, followed by extraction with hot 70 per cent alcohol. E. D, followed by extraction with cold water. In this particular case, growth was obtained following extraction with 70 per cent alcohol, and the strength, density and rate of growth were improved by extraction with cold water.

Fig. 2. Shows the effect of adsorbed cations on mycelial growth in the case of compost no. 3. The compost was treated with chloride solutions as described on p. 358. D. Contains adsorbed Na. E. Contains adsorbed K. R. Contains adsorbed Mg. S. Contains adsorbed Ca. The weak, thin mycelial growths with Na, K and Mg are in marked contrast to the strong, dense growth with Ca.

### 376 *Environment and Nutrition of Cultivated Mushroom*

- Fig. 3. Is of compost no. 6, a short, highly decomposed greasy compost. Treatments A-E are the same as in Fig. 1; they failed to bring about growth. F. Extracted with cold water. G, F, followed by flocculation with  $N/20$   $\text{CaCl}_2$ . H. Soaked in  $N/20$   $\text{CaCl}_2$  and rinsed with water. The effect on mycelial growth of flocculating the colloids—insoluble as well as soluble—in composts with calcium ions is strikingly shown; whereas on the unflocculated compost there is no growth at all, flocculation produces a strong, dense mycelium.
- Fig. 4. Is of compost no. 7 and, like Fig. 3, shows the effect of dispersion on mycelial growth. A. Untreated compost—weak hyphae, medium density. B. Extracted with cold water—strong hyphae, medium density. C. B, followed by flocculation with  $N/20$   $\text{CaCl}_2$ —strong hyphae, dense mycelium. D. Extracted with  $N/20$   $\text{CaCl}_2$ —strong hyphae, dense mycelium. E. 1 per cent of  $\text{CaSO}_4$  mixed in—strong hyphae, dense mycelium.
- Fig. 5. Shows the effect on mycelial growth of the water extract of compost no. 1, details on p. 369. A, E. Full quantity water extract. B, F. Three-quarters water extract. C, G. One-quarter water extract. D, H. No water extract. A-D. On paper with solution A. E-H. On paper with solution B. A falling off in the density and strength of growth with increasing quantities of water extract is very well shown; also the improved density of growth due to ammonium nitrate (A-D).

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Fig. 1.

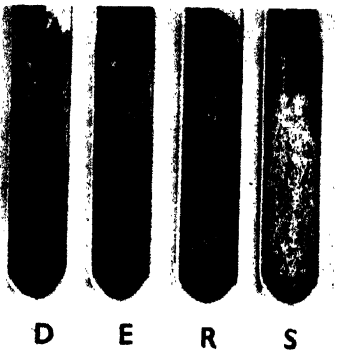


Fig. 2.



Fig. 3.



Fig. 5.



Fig. 4.



# THE EFFECT OF LOW-TEMPERATURE GRAIN PRE-TREATMENT ON THE DEVELOPMENT, YIELD AND GRAIN OF SOME VARIETIES OF WHEAT AND BARLEY

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## INTRODUCTION

PREVIOUS experiments conducted at Cambridge have demonstrated that low-temperature grain pre-treatment may affect the early development of certain varieties of cereals in addition to causing an acceleration in the time of earing (1). The most striking effect on early development was seen in the number of tillers produced, and a tentative suggestion was made that this might also affect the number of ears per plant surviving at harvest. It seemed probable that the yield and quality of the grain might be affected by such fundamental changes in early development, as has been claimed by certain Russian investigators (2). Any such effect on yield and quality is of greater importance than acceleration in earing in this country, and it was decided to investigate this problem in wheat and barley.

An experiment was accordingly designed to make a developmental study of the effect of low-temperature pre-treatment. It was hoped by this means to make a full analysis of the effects of the treatment on important agronomic and economic characters which could justify its use on a practical scale. The characters chosen were tillering, ear survival, yield, 1000-grain weight and nitrogen content of the grain, the yield being further analysed in terms of weight of grain per plant and per ear. In addition, by choosing spring and winter varieties, it would be possible to compare the behaviour of spring-sown winter varieties which had been pre-treated, with spring varieties, and by this means to test the possibility of substituting the latter by the former on a practical scale.

The investigation has also proved useful in the study of varietal behaviour in relation to tillering, ear survival and yield, quite apart from any low-temperature pre-treatment effects. Therefore, in the account of the experiments given below, the pre-treatment effects are discussed

separately from the varietal developmental and yield characters in order to avoid confusion and to emphasize certain important points with regard to varietal behaviour. It has also been thought advisable to keep the barley and the wheat experiments separate although the procedure adopted was identical in both cases.

#### TECHNIQUE AND EXPERIMENTAL LAY-OUT

All the varieties, with the exception of the spring wheat Red Marvel, had been used in previous experiments and certain facts were known concerning their response to low-temperature pre-treatment. The wheat varieties were the two winter varieties Yeoman II and Joss IV, and the spring variety Red Marvel. The barley varieties were all two-row forms (*Hordeum distichum*), and consisted of the winter varieties Tschermak's and Stadler's A, and the spring variety Spratt-Archer.

The low-temperature pre-treatment was conducted by placing wetted grain in low-temperature chambers. Forty parts by weight of distilled water were added to the winter varieties, and thirty-two parts to the spring varieties. The germinating dishes with the wetted grain were allowed to stand in the laboratory for 24 hours before being removed to the low-temperature chambers, where the winter varieties were exposed to a temperature of 3° C. for 47 days, and the spring varieties to a temperature of 5° C. for 10 days. The control samples were subjected to the same treatment of soaking in distilled water for 24 hours previous to their sowing with the treated samples, which were taken directly from the low-temperature chambers on the day that the sowing was made.

The grain was sown in two separate 6 × 6 Latin squares on 10 March 1936, the whole of the sowing being accomplished in a few hours after removal from the low-temperature chambers. The wheat and barley were, of course, included in separate Latin squares, and six plots each consisting of 120 grain were sown to each control and treated variety sample. The grain was dibbed in rows 6 in. apart with 2 in. between the plants, and each trial was surrounded by a double discard row of spring wheat.

The data has been treated according to the normal analysis of variance method, but the figures in the text have been reduced to a minimum. For each set of data the interaction effect, treatment effect, and variety effect were calculated. As far as the effect of the treatment is concerned, only the first two need be considered, and a discussion of the variety effect is reserved for special consideration at the end of each section.

## A. BARLEY

(1) *The effect of low-temperature pre-treatment on tillering and ear survival at harvest*

Two tiller counts were made before shooting commenced, the first on 27 April which was 48 days after sowing, and the second on 13 May which was 64 days after sowing. The number of surviving ears was counted at harvest, the criterion of a "surviving ear" being the presence of germinable grain. The three sets of observations should yield data on three distinct and important characters in the development of the cereal plant, i.e. the rate of early tillering; the maximum number of tillers produced; the survival of tillers as ears at harvest, with a consequent measurement of the significance of tillering in relation to the number of ears produced.

In Tables I, II and III below the data for these three counts are given. The averages of the six plots of each treated and control sample are given, together with the sum and difference between these figures for each

Table I. *First tiller count. Average tillers per plant*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	1.465	1.865	1.981	0.077
Treated	1.573	1.383	1.791	
Total	3.038	3.248	3.772	0.108
Difference	0.108	-0.482	-0.190	

Table II. *Second tiller count. Average tillers per plant*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	4.52	7.43	5.12	0.133
Treated	4.56	4.89	2.97	
Total	9.08	12.32	8.09	0.189
Difference	0.04	-2.54	-2.15	

Table III. *Harvest count. Average ears per plant*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	2.48	1.44	1.44	0.083
Treated	2.57	1.54	1.31	
Total	5.05	2.98	2.75	0.117
Difference	0.09	0.10	-0.13	

variety. In addition, the Standard Errors are included in the last column of the tables. All the other tables given in this paper are calculated in the same manner.



In the first tiller count the average vernalization effect was significant ( $P < 0.01$ ), there being a decrease in the number of tillers due to the treatment. But the interaction effect was also significant ( $P < 0.01$ ) and it was therefore possible to study individual varietal behaviour. Both Tschermak's and Stadler's A show a decrease in tiller production in the treated plots, the former being affected to a greater degree than the latter, while Spratt-Archer is unaffected.

The second tiller count shows a very large average treatment effect, there being a significant decrease in tiller production ( $P < 0.01$ ). Analysis of the significant interaction effect ( $P < 0.01$ ) once more shows that Tschermak's is subject to the greatest tiller reduction, but Stadler's A is also very strongly affected. Spratt-Archer remains unaffected by the treatment. The outstanding fact brought out by these figures is the enormous reduction in tiller number of the two winter varieties due to low-temperature treatment.

The harvest ear count did not give any significant treatment or interaction effects, and the slight increases shown by Spratt-Archer and Tschermak's, and the slight decrease of Stadler's A, must therefore be considered as insignificant.

(2) *The effect of low-temperature pre-treatment on yield*

In Tables IV, V and VI below are given the average yield per plot, per plant and per ear for the control and treated samples of each variety.

Table IV. *Average yield per plot in grams*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	253	206	143	16.10
Treated	276	181	159	
Total	529	387	302	21.33
Difference	23	- 25	16	

Table V. *Average yield per plant in grams*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	2.33	1.99	1.42	0.136
Treated	2.54	1.86	1.57	
Total	4.87	3.85	2.99	0.192
Difference	0.21	- 0.13	0.15	

Table VI. *Average yield per ear in grams*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	0.96	1.37	0.98	0.044
Treated	0.97	1.20	1.19	
Total	1.93	2.57	2.17	0.063
Difference	0.01	- 0.17	0.21	

No significance could be attached to either the average treatment effect or the interaction effect in the gross yields per plot. In fact the increase in yield observable in the Spratt-Archer treated plots may be traced to the exceptional luxuriance of one particular plot, and the decrease in yield of the treated plots of Tschermak's can be attributed to some extent to the higher mortality of plants amongst the treated plots. There was no observable explanation for the increase in yield of Stadler's A.

The yields per plant vary in the same direction as the yields per plot as would be expected, but still no significant differences were found. Once more the higher yield of the treated Spratt-Archer plants is due to the one plot mentioned above.

In the light of what has been said above with regard to the yields per plot and per plant, the figures for the yield per ear are of interest. The average treatment effect was not significant, but the interaction effect was significant ( $P < 0.01$ ) and the behaviour of each variety may be studied. Spratt-Archer shows no effect whatever, while Tschermak's shows a significant decrease, and Stadler's A a significant increase. Therefore the slight effects seen in the yields per plot and per plant in these two latter varieties can be attributed to the significant effect on the yield per ear.

### (3) *The effect of low-temperature pre-treatment on the grain*

The 1000-grain weights and the nitrogen content of the grain measured as a percentage of the dry weight are given in Tables VII and VIII below. No significance was obtained for the average treatment effect or the interaction effect on the nitrogen percentage, but both the treatment

Table VII. *Average 1000-grain weight in grams*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	43.34	57.82	60.62	0.468
Treated	44.08	56.34	65.28	
Total	87.42	114.16	125.90	0.663
Difference	0.74	- 1.48	4.66	

Table VIII. *Average nitrogen as percentage of dry weight*

	Spratt-Archer	Tschermak's	Stadler's A	S.E.
Control	1.393	1.790	2.002	0.029
Treated	1.399	1.803	1.965	
Total	2.792	3.593	3.967	0.041
Difference	0.006	0.013	- 0.037	

effect ( $P < 0.01$ ) and the interaction effect ( $P < 0.01$ ) were significant for the 1000-grain weight. Analysis of the interaction effect showed that Spratt-Archer was unaffected, while the 1000-grain weight had been decreased in Tschermak's and very greatly increased in Stadler's A.

### *Discussion*

The discussion of the results on the effect of low-temperature pre-treatment is perhaps most easily treated by dealing with each variety separately because of the individualistic varietal behaviour. The spring variety Spratt-Archer was not subjected to the same treatment as the two winter varieties, but previous investigations had shown that this variety was unaffected by various treatments which had very marked effects on the winter varieties. The view is held by the Russian workers that spring varieties will respond only to shorter exposures of a higher temperature than those suitable for winter varieties. Accordingly, Spratt-Archer was subjected to the type of treatment advocated for spring varieties, but even so no significant effect was obtained in any of the observations made in the present experiment. It is possible that the treatment was not suitable for obtaining any response, because it has been shown in previous experiments that varieties are strongly individualistic in their requirements and response. Investigations with other treatments involving different temperatures and lengths of exposure might be successful in producing tangible effects.

Tschermak's, which is a two-row winter barley of vigorous growth in the early stages of development, suffered a very strong inhibition of tiller production, but the leaves were larger in the treated plants. In spite of the great reduction in the number of tillers due to the treatment there was no marked difference in the time of inception of the shooting phase of development, but as shooting progressed it became obvious that the control plants would be later in coming into ear than the treated, and there was actually about 7 days difference in the time of earing. As was pointed out previously, the great difference in tiller production between the treated and control plants was not accompanied by a significant difference in the number of ears surviving at harvest, although the lower tillering treated plants tended to produce slightly more ears. But this slight increase in ear number was not associated with any increase in the total yield per plot or per plant; there was, in fact, a tendency towards lower yields in both cases. These decreases could be traced directly to the significant lowering of the yield per ear which was probably due to the smaller 1000-grain weight of the grain from the treated plants. No

treatment effect was obtained in the nitrogen percentage in spite of the effect on the 1000-grain weight.

In the early stages the winter variety Stadler's A was affected in the same way as Tschermak's, there being a strong decrease in the number of tillers produced in the treated plants, but no significant effect resulted in the number of surviving ears. In contrast to Tschermak's, however, the number of ears in the treated plants of Stadler's A tended to be lower than in the control plants. From this point the behaviour of Stadler's A was exactly the reverse of Tschermak's, the yield per plot, per plant and per tiller being higher in the treated samples, although only the yield per tiller difference was significant. This increase in yield appears to have been due to a large extent to the very great increase in the 1000-grain weight of the grain from the treated plants, but once more this effect on the 1000-grain weight was not accompanied by any significant change in the nitrogen content.

The behaviour of these two varieties emphasizes very strongly how the tillering of any variety may be strongly affected without interfering with the normal economy of the plant in relation to ear production. Apparently the effect of low temperature on winter varieties is most strongly seen in the curtailment of tiller production, and it is well known that spring sowing of winter varieties tends to encourage excessive vegetative growth measured in terms of tillers.

#### (4) *Varietal behaviour in relation to the characters studied*

The three varieties studied in this experiment are of considerable interest in their developmental behaviour, yield and grain characters quite apart from any low-temperature pre-treatment effects. In the calculation of each set of data the variety effect was always easily significant at the 1 per cent point, and in most cases completely overshadowed the treatment and interaction effects. A brief account of this aspect of the study will now be given.

Referring back to Tables I, II and III, the behaviour of each variety with regard to tillering and ear survival may be compared. Stadler's A tillers most abundantly in the early stages, while Tschermak's is very little inferior in this respect. Both of these varieties are considerably more vigorous in tiller production than Spratt-Archer, and the second tiller count emphasizes this difference even more strongly than does the first count. But at maximum tillering Tschermak's is considerably above Stadler's A, which does not maintain its early vigour of growth, but is still superior in tiller number to Spratt-Archer. However, in spite

of the high rate of tillering of Tschermak's and Stadler's A the number of ears surviving at harvest is considerably below that of Spratt-Archer, while even the comparatively large difference in maximum tiller production of the two former varieties is not accompanied by any difference in their ear survival.

The effect of the high ear survival of Spratt-Archer is seen clearly in Tables IV and V where the yields per plot and per plant of this variety easily exceed those of the two winter varieties. Further, in spite of the fact that Stadler's A produces as many ears as Tschermak's, its yields are considerably below the latter variety. The explanation of this is in the low yield per ear of Stadler's A compared with Tschermak's which is easily the highest in this respect. When the figures for the 1000-grain weight given in Table VII are considered it may be seen that Stadler's A leads in this respect, with Tschermak's second and Spratt-Archer considerably lower than either. But in spite of this high 1000-grain weight of Stadler's its yield per ear is only equal to Spratt-Archer.

From these figures, therefore, yield appears to be primarily due to the number of ears surviving at harvest. Spratt-Archer gives the highest gross yields because of its superiority in this respect, which ensures a high yield per plant. Tschermak's is second to Spratt-Archer in its yield per plot and per plant, and its high 1000-grain weight which raises the yield per ear to first place cannot compensate for the lack of one ear per plant compared with the latter variety. The only case where Stadler's A is superior to the other two varieties is in the 1000-grain weight, but although this is responsible for a yield per ear equal to Spratt-Archer, its inferiority in all other respects entails a lower yield per plot. It is interesting that although this variety has a ear survival equal to, and a 1000-grain weight superior to, Tschermak's the latter variety out-yields it in all respects. This is presumably due to the larger number of grain per ear in Tschermak's. Therefore, the comparative effects on yield of ear survival, number of grain per ear, and 1000-grain weight may be inferred from the figures given in this experiment.

The relationship of the nitrogen content of the grain to the other characters is of considerable importance from the point of view of malting barley. Spratt-Archer possesses the lowest nitrogen figure, Stadler's A the highest, and Tschermak's is intermediate. It would appear, therefore, that the nitrogen content of the grain is closely associated with the number of ears at harvest and the yield per plant and per tiller. The low nitrogen percentage of Spratt-Archer is due primarily to the first two characters, i.e. high number of ears and yield per

plant, while the higher nitrogen percentage of Stadler's A compared with Tschermak's in spite of their equal ear survival, is due to the fewer number of grain per ear of the former. It may also be seen that the nitrogen percentage is positively correlated with the 1000-grain weight, and the key to the whole question of nitrogen percentage probably lies in the number of grains per plant. Because there is a very definite limit to ear size, this may be interpreted in terms of ears per plant, and varieties with high ear survival will tend to produce low nitrogen percentage grain. Six-row barleys are in general characterized by grain of low nitrogen content in spite of the fact that ear survival tends to be low in these forms. The reason for this may well lie in the fact that six-row barleys have a high number of grain per ear, and consequently the nitrogen in each grain is lower than it would be if the ears possessed fewer grain.

In conclusion, the inability of tillering capacity to give a measure of yield may be emphasized. Yield is strongly dependent on the number of ears per plant, and this latter character is in no way dependent on the tillering propensities. In plant selection for high yield, tiller counts are unnecessary; the true basis of selection should rest on the number of ears surviving at harvest accompanied by an analysis of the yield per ear.

## B. WHEAT

### (1) *The effect of low-temperature pre-treatment on tillering and ear survival at harvest*

Tiller counts were made 48 and 64 days after sowing as was the case with barley, and the number of surviving ears was counted at harvest. The observations, therefore, are strictly comparable with those made on the barley varieties and do not require any discussion. The data for the three counts are summarized in Tables IX, X and XI.

No significant interaction effect was obtained in the first tiller count, but the average treatment effect was significant ( $P < 0.05$ ), due to the reduction in tiller number of Yeoman II and Joss IV. This reduction in the number of tillers of Yeoman II and Joss IV was very much more marked in the second count, where the interaction effect was significant ( $P < 0.01$ ), in addition to the average treatment effect. The figures show that Joss IV was most strongly affected, Yeoman II suffered a smaller reduction, while Red Marvel showed a small reduction which had not been apparent in the first count.

The harvest ear count did not give any significance to the interaction

effect or the average treatment effect, so that in spite of the great difference between the treated and control plant in tiller number, no effect was obtained on the number of ears produced.

Table IX. *First tiller count. Average tillers per plant*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	2.23	2.84	1.50	0.092
Treated	2.01	2.46	1.50	
Total	4.24	5.30	3.00	0.130
Difference	-0.22	-0.38	0.00	

Table X. *Second tiller count. Average tillers per plant*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	6.74	8.41	2.49	0.063
Treated	4.68	5.01	2.01	
Total	11.42	13.42	4.50	0.089
Difference	-2.06	-3.40	-0.48	

Table XI. *Harvest count. Average ears per plant*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	2.02	2.35	2.21	0.078
Treated	2.19	2.39	2.22	
Total	4.21	4.74	4.43	0.110
Difference	0.17	0.04	0.01	

(2) *The effect of low-temperature pre-treatment on yield per plot, per plant and per ear*

The figures for yield per plot, per plant and per ear are summarized in Tables XII, XIII and XIV below.

In spite of the general trend to an increased yield per plot in the treated samples, neither the average treatment effect, nor the interaction effect, was significant. The treatment effect barely missed significance at the 5 per cent point, and it should be pointed out that the number of plants in the six treated plots of Yeoman II was over 10 per cent less than in the control plots. It is highly probable that if there had not been this high loss of plants there would have been a significant increase in yield in the treated plots. This supposition is borne out by the fact that there was a significant average treatment effect in the yield per plant ( $P < 0.05$ ). There was, however, no interaction effect, and the varietal behaviour could not be further analysed. The yield per ear analysis did not give significance for either the average treatment effect or the interaction effect, so that the yield effect was only to be seen in yield per plant. It is perhaps worth mentioning that the average

treatment effect very nearly approached significance, and it may be seen from Table XIV that each variety showed a tendency to an increased yield in the treated samples.

Table XII. *Yield per plot in grams*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	173	166	218	6.4
Treated	182	177	231	
Total	355	343	449	9.00
Difference	9	11	13	

Table XIII. *Yield per plant in grams*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	1.65	1.79	2.17	0.113
Treated	1.93	2.08	2.27	
Total	3.58	3.87	4.44	0.160
Difference	0.28	0.29	0.10	

Table XIV. *Yield per ear in grams*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	0.808	0.743	0.981	0.031
Treated	0.877	0.788	1.013	
Total	1.685	1.531	1.994	0.043
Difference	0.069	0.045	0.032	

### (3) *The effect of low-temperature pre-treatment on the grain*

Tables XV and XVI give the data for 1000-grain weight and nitrogen content of the grain. A significant average treatment effect at the 5 per cent point showed that the 1000-grain weight had been increased by the treatment. Although there was no significant interaction effect, it may be seen that Joss IV was largely responsible for the treatment effect, the other two varieties being only slightly affected.

The interaction effect for the nitrogen percentage was significant at the 1 per cent point, but there was no average treatment effect. The figures show that Joss IV was subjected to an increase and Red Marvel to a decrease in nitrogen percentage, while there was no apparent effect on Yeoman II.

Therefore the treatment was responsible for increasing the 1000-grain weight and nitrogen percentage of the grain in Joss IV and decreasing the nitrogen percentage in Red Marvel. Joss IV was the most susceptible to the treatment, Red Marvel showed a slight response, and Yeoman II was entirely unaffected.



Table XV. *Thousand-grain weight in grams*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	43.47	41.07	51.99	1.113
Treated	43.64	45.65	52.46	
Total	87.11	86.72	104.45	1.60
Difference	0.17	4.58	0.47	

Table XVI. *Nitrogen content as percentage of dry weight*

	Yeoman II	Joss IV	Red Marvel	S.E.
Control	1.997	1.857	1.965	0.0246
Treated	2.002	2.013	1.892	
Total	3.999	3.870	3.857	0.0175
Difference	0.005	0.156	-0.073	

*Discussion*

The behaviour of the two winter wheat varieties has much in common with the two winter barley varieties with regard to tillering and ear survival. The effect of the treatment was to reduce the number of tillers considerably, without affecting the number of ears in the case of both cereals, and it is interesting to see that the highest tillering varieties—Tschermak's and Joss IV—suffered the greatest reduction. The wheat investigation therefore supports the view already put forward that low-temperature exposure is responsible for curtailing tiller development in winter varieties, and that the tendency for such varieties to run to excessive vegetative growth when sown in the spring is due to the absence of the low temperature.

The spring wheat variety Red Marvel received the same treatment as Spratt-Archer, but it differed from the latter variety in showing a slight effect on tiller production. But the reduction of tiller number was not accompanied by any effect on the ear survival, and the two experiments show that in spite of the individual and different behaviour of the various varieties in relation to tiller production, low-temperature pre-treatment had no significant effect on the number of ears surviving at harvest.

The morphological differences between the control and treated plants were not very striking, but in Yeoman II and Joss IV the treated plants were a little larger and more erect. Definite acceleration in the time of earing was apparent in Joss IV where the treated plants were eight or nine days earlier than the controls. There was only a 1- or 2-day difference between the treated and control plants of Yeoman II, while Red Marvel showed no effect of the treatment in this respect. There were no differences

in the length of the straw between the treated and control plants of Yeoman II and Red Marvel, but the treated plots of Joss IV appeared shorter and more uniform in height than the controls, and colour changes during ripening were more rapid in the former than the latter.

The treatment effects on the yield figures were not so pronounced in the wheat as they were in the barley, the only significant effect being due to the treatment on the yield per plant. In no case was there a significant interaction effect and consequently varietal behaviour cannot be analysed. It is possible that if the mortality of the treated plants of Yeoman II had not been so high, there would have been a more pronounced increase in the treatment effect on the yield per plot, but it is interesting that no effect on the yield per ear was obtained as was the case in the barley experiment where a significant interaction showed an increase in Stadler's A and a decrease in Tschermak's. This was bound up with a corresponding effect on the 1000-grain weight of these two varieties where the interaction effect was significant. The wheat varieties do not show this significant interaction effect on the 1000-grain weight, and although the increase in yield per plant in Joss IV must be due largely to the increased 1000-grain weight, the corresponding increase in yield per plant of Yeoman II can have no such explanation, but must be related to the small increase in the number of ears surviving at harvest and to an increase in the size of the ear which is shown by the higher yield per ear of the treated plants.

The effect of the treatment on the nitrogen content of the grain in these three varieties is interesting in that each variety behaved differently. Joss IV, which was affected to the greatest degree, showed a distinct increase in the nitrogen percentage, which, it may be noted, was associated with the increase in 1000-grain weight. The decrease in the nitrogen percentage of Red Marvel was, however, not associated with any such effect on the grain weight, while Yeoman II was unresponsive in both respects.

This experiment, therefore, does bring forward evidence to show that the low-temperature grain pre-treatment may have a small effect on the yield and quality of the grain of some wheat varieties. Rough experiments on the grain of Joss IV did not show any increase in the yield of gluten accompanying the increased nitrogen percentage, but no tests were done to investigate the bread-making quality. It is not probable that the effects of low-temperature pre-treatment of the dimensions described in this experiment would ever be considered of sufficient importance to warrant the use of the treatment in practical agriculture.

Later sowings would in all probability yield more striking results, but the object of the experiments described in this paper was to test the efficacy of the treatment on a normal spring sowing. Low-temperature pre-treatment is only regarded as a temporary expedient to be used in areas for which suitable varieties of cereals have not as yet been developed. There are cereal growing areas in the world which are backward or under developed where extreme climatic conditions give the treatment a definite practical value, but the conditions in this country and the varieties at the disposal of the grower render it at present somewhat superfluous.

(4) *Varietal behaviour in relation to the characters studied*

The wheat experiment resembled the barley experiment in that the variety effect was significant in each set of observations, and in some cases was the only significant result obtained. The developmental behaviour of each variety, with its effect on the yield, and the differences shown in the grain types may be studied therefore.

Joss IV proved itself to be the highest tillering variety. In the first count where significance was reached at the 1 per cent point it showed a clear superiority over Yeoman II, which in turn was superior to Red Marvel (Table IX). The second count emphasized these differences and the great discrepancy between the maximum tillering of winter and spring varieties is once more demonstrated ( $P < 0.05$ ) (Table X). But the very high mortality of tillers in winter varieties when they are spring sown is illustrated by the figures in Table XI which shows the ears surviving at harvest. Joss IV retained the highest number of ears, but it suffered the greatest percentage loss of tillers, while Red Marvel showed an extraordinarily high tiller survival as ears at harvest, by means of which it proved superior to Yeoman II in its average number of ears per plant. But perhaps one of the most striking things concerning the figures for the average number of ears per plant is the similarity of the three varieties in this respect, which resulted in significance being only barely reached at the 1 per cent point.

The yield per plot figures show a clear superiority for Red Marvel ( $P < 0.01$ ), but it should be emphasized that Joss IV suffered a higher plant mortality than the other two varieties which resulted in the total number of plants for the six control plots being approximately 10 per cent less than Yeoman II and approximately 7 per cent less than Red Marvel. In spite of the greater number of plants per plot in Yeoman II its yield was lower than that of Red Marvel (Table XII).

The higher yielding properties of Red Marvel are further emphasized in the figures for yield per plant ( $P < 0.01$ ) and yield per ear ( $P < 0.01$ ) shown in Tables XIII and XIV. In both cases Red Marvel gave distinctly higher yields than either of the winter varieties which reverse their positions in the two tables, Joss IV showing a slight but insignificantly higher yield per plant than Yeoman II, which is superior to the former variety in yield per ear. The explanation for the relative positions of the three varieties in yield per ear may be found in their 1000-grain weights (Table XV). Here Red Marvel was strikingly superior to the two winter varieties, while Yeoman II was superior to Joss IV in this respect ( $P < 0.01$ ).

The nitrogen content of the grain was marked by a strong similarity between the three varieties, and the variety effect only showed significance at the 5 per cent point. Yeoman II and Red Marvel must be considered as having grain of equal nitrogen percentage while Joss IV is inferior in this respect, although the difference between this variety and the other two is not large.

Therefore in this experiment the spring variety Red Marvel has shown a distinctly higher yielding capacity than either of the two winter varieties Yeoman II or Joss IV. This higher yielding capacity was due primarily to the superior 1000-grain weight which ensured a greater yield per plant in spite of the tendency to a lower ear production than Joss IV. The low-temperature treatment tended to increase the yields of each variety although no significance was reached, but even so, Red Marvel continued to outyield the two other varieties, and the relative positions of the three varieties is the same whether the grain has been pre-treated or not. There appears to be little to choose between Yeoman II and Joss IV as far as yield is concerned, because although Joss IV developed more ears at harvest, the yield per ear was higher in Yeoman II, so that there was little difference in the yield per plant. Both the wheat and the barley experiments have failed to demonstrate any superiority of pre-treated winter varieties over spring varieties, in fact in both cases the latter outyielded the former. This appears to be due to the failure of the treatment to stimulate a higher ear survival in the winter varieties which easily produce more tillers than the spring varieties. It seems evident that spring-sown winter varieties are characterized by higher tillering and lower percentage ear survival than spring varieties, but unless a treatment can be found which will induce higher ear survival in the higher tillering varieties there is little likelihood of any advantage to be gained from their use in preference to a lower tillering spring variety.

In conclusion it may perhaps be emphasized once more that this study of the developmental and mature plant characters of the three wheat varieties demonstrates the importance of such studies in elucidating varietal yield problems. Such studies are of particular importance and significance in plant breeding where the behaviour of potential parents should be examined thoroughly with respect to those characters which affect yield and quality before any attempts are made to hybridize. It is only by detailed examination that suitable parents can be chosen which will be most liable to give the best combinations in hybrid progenies. This is particularly the case in breeding for high yield where it is essential first to understand the behaviour of the parents with regard to all those characters which may contribute to high yielding capacity.

#### SUMMARY

1. The effect of low-temperature grain pre-treatment on two winter varieties of wheat and two winter varieties of barley is seen in a very definite reduction in the number of tillers produced, which, however, had no subsequent effect on the number of ears surviving at harvest. Very little effect resulted from the treatment of spring varieties of wheat and barley in the number of tillers produced, although the spring wheat variety did show a slight reduction.

2. Gross yields per plot were unaffected by the treatment in all the varieties, but the yields per plant were affected in the wheat experiment, and the yield per ear in the barley experiment. There was a general increase in the yield per plant of all wheat varieties, while in the yield per ear one barley variety was unaffected, another showed an increase, while the third suffered a reduction.

3. The grain from treated plants was affected in 1000-grain weight and nitrogen content in the wheat experiment, while only the 1000-grain weight suffered any change in the barley experiment. Only one wheat variety showed an increase in 1000-grain weight, while each of the three varieties responded differently with regard to the nitrogen content of the grain. Similarly, each of the three barley varieties was affected differently with regard to 1000-grain weight, one being unaffected, another showing a decrease, while the third showed an increase.

4. Analysis of the behaviour of the varieties with regard to tillering and ear production showed clearly that the maximum of tillers produced bears little relation to the number of ears at harvest. In the barley varieties high yield and low nitrogen were associated with the largest number of ears, which in turn was associated with the lowest tillering.

In the wheat varieties high yield was associated with high 1000-grain weight. In both experiments the spring varieties outyielded the winter varieties whether the latter had been treated or not.

The writer wishes to thank Messrs M. C. Buck, J. D. Palmer and K. P. Hedge for carrying out the tiller counts and supervising the threshing and weighing of the samples.

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# THE BODY PROPORTIONS OF DIFFERENT BREEDS OF BACON PIGS

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(With Plates V and VI and Seven Text-figures)

## CONTENTS

	PAGE
Introduction . . . . .	394
Material and methods . . . . .	395
The shrinkage from live weight to carcass and bacon weights . . . . .	399
The optimum proportions—market requirements . . . . .	404
The changes in the proportions of the side with increase in weight:	
(a) General . . . . .	406
(b) Breed differences . . . . .	414
The changes in proportions with increase in length . . . . .	420
Effect of sex on body proportions . . . . .	428

## INTRODUCTION

WHILE much work has been done on the body measurements and proportions of bacon-pig carcasses in Denmark<sup>(3)</sup>, Sweden<sup>(1)</sup>, Holland<sup>(17)</sup>, Norway<sup>(15)</sup>, etc., this has been limited to comparatively few breeds and types. Hence the results have not been such as to indicate what the differences of growth and proportions of the different types of pigs are. In this country we have a variety of types (pork, dual purpose, and bacon) of pigs, and so this investigation aims at elucidating the principles of growth which underlie the development of the bacon type by comparison of their growth with those of other types.

The proportions of the body in the pig change as the pig grows up, but how far these changes in proportions are necessarily associated with changes in weight has not yet been accurately determined. Since it is probably in this relationship that the breeds and types differ, i.e. in the relation between body increases in weight and changes in body proportions, these changes have been investigated. Photographs and diagrams to illustrate this have already been published in a popular pre-

liminary account of this investigation (7); that publication will serve as an introduction to the present paper.

While the different breeds are described below in terms of changes in proportions with increase in body weight, it is not suggested that all the individuals of a breed are necessarily similar, and there may be considerable variations from the average type of the breed as described here. It is considered, however, that these body proportions constitute some of the main commercial qualities of the breed (i.e. for pork or bacon purposes, according to weight), and that it is the breeding to these which constitutes "breeding to type". The showyard standards of type adopted by the Breed Society will depend on whether the breed sets out to supply the pork or bacon market, or to be dual purpose. The facts given below should be of assistance to those who are attempting to form these standards.

The results of crossing two different types are also shown below. Not only are these useful in showing the mode of inheritance in first crosses between different breeds, but, since within each breed different types exist, the results of these crosses will show the breeder the means by which he can correct deficiencies, from a bacon point of view, in his own strain by the use of animals of rather different type from another strain within the breed.

The facts given in this paper also supply data which may be useful to the bacon industry.

#### MATERIAL AND METHODS

The material used consisted of cured and smoked sides of bacon exhibited at the London Dairy Show from 1922 to 1931 inclusive (with the exception of the year 1926). This was before any standards were fixed for bacon pigs; after the time when standards were fixed, pigs unsuited for producing bacon were eliminated from the exhibition, and so perhaps the results would not be so representative of the breeds as a whole.

The breeder selected the pigs and sent them on a certain date to Messrs Harris's Bacon Factory at Calne where they were all weighed, slaughtered and cured under uniform conditions. All the particulars about the sides were known—breed or cross, sex, age, live weight, carcass weight and bacon weight<sup>(12)</sup>—and, during the Show, measurements were taken of each side.

The following measurements (in mm.) were taken to describe as far as possible the type of the side; the exact points of measurements are illustrated in Plate V:



Table I. The live and carcass weights required to produce bacon sides of a given weight

[illegible]

**(b) Percentage of live weight**

No.	No.	No.	No.	No.	No.	No.	No.
30-34	—	—	—	—	—	—	—
35-39	—	—	—	—	—	—	—
40-44	7	77·8	52·0	—	—	—	—
45-49	19	77·1	55·0	—	—	—	—
50-54	78	78·1	57·8	13	79·2	55·4	8
55-59	86	79·5	59·0	12	78·5	56·2	3
60-64	31	78·4	61·0	24	75·9	55·4	18
65-69	2	77·7	57·0	15	80·9	60·2	2
70-74	5	79·1	60·6	16	81·1	60·4	—
75-79	—	—	—	4	82·8	61·2	—
80-84	3	79·5	60·8	9	85·1	66·6	—
85-89	—	—	—	3	83·0	65·0	—
90-94	5	70·2	62·0	—	—	—	—

Table I (cont.)

Breed ... Bacon side weight	Middle White			Large White × Large Black			Large White × Berkshire			Large White × Middle White			All Breeds and Crosses			Carcass weight minus bacon sides weight	Actual average bacon side weight	
	Live	Car.	Bacon sides	Live	Car.	Bacon sides	Live	Car.	Bacon sides	Live	Car.	Bacon sides	Live	Car.	Bacon sides			
(a) Weight (lb.)																		
30-34	—	—	—	—	—	—	—	—	—	—	—	—	139	105	74	31	37.0	
35-39	140	103	74	—	—	—	—	—	—	—	—	—	156	120	85	35	42.5	
40-44	159	120	86	—	—	—	—	—	—	—	—	—	167	130	94	36	47.0	
45-49	176	132	98	—	—	—	—	174	133	97	160	126	99	167	130	94	36	47.0
50-54	177	138	104	189	145	103	179	139	104	184	143	104	185	145	105	40	52.5	
55-59	208	159	118	201	155	115	193	154	113	198	154	116	198	155	115	40	57.5	
60-64	—	—	—	213	163	123	209	168	127	218	172	127	209	164	124	40	62.0	
65-69	206	168	135	—	—	—	—	—	—	218	174	132	223	179	134	45	67.0	
70-74	—	—	—	—	—	—	—	—	—	—	—	—	235	190	143	47	71.5	
75-79	—	—	—	—	—	—	—	—	—	—	—	—	246	201	154	47	77.0	
80-84	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
85-89	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
90-94	—	—	—	—	—	—	—	—	—	—	—	—	261	214	168	46	84.0	

(b) Percentage of live weight

No.	No.		No.		No.		No.		No.		No.		Carcass weight minus bacon sides weight				
	Car.	Bacon sides	Live	Car. cass	Bacon sides	Live	Car. cass	Bacon sides	Live	Car. cass	Bacon sides	Live	Car.	Bacon sides	As % of live weight	As % of carcass weight	
30-34	—	—	—	—	—	—	—	—	—	—	—	—	17	75.5	53.2	22.3	28.5
35-39	6	73.6	52.8	—	—	—	—	—	—	—	—	—	25	76.9	54.5	22.4	29.2
40-44	4	75.2	53.6	—	—	—	—	—	—	—	—	—	25	76.9	54.5	22.4	29.2
45-49	2	75.0	55.6	—	—	—	—	—	—	—	—	—	25	76.9	54.5	22.4	29.2
50-54	9	78.0	59.0	6	77.0	54.4	7	77.7	57.8	6	77.6	56.5	178	78.4	56.8	18.4	27.6
55-59	2	76.4	56.7	20	77.3	57.2	4	79.9	58.4	9	77.9	58.6	214	78.3	58.1	20.2	25.5
60-64	—	—	—	10	76.4	57.8	6	80.1	60.8	2	79.1	58.2	117	78.5	59.3	19.2	24.4
65-69	3	81.3	65.4	—	—	—	—	—	—	2	79.8	60.6	38	80.3	60.1	20.2	25.1
70-74	—	—	—	—	—	—	—	—	—	—	—	—	29	80.9	60.9	20.0	24.7
75-79	—	—	—	—	—	—	—	—	—	—	—	—	19	81.7	62.6	19.1	23.4
80-84	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
85-89	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
90-94	—	—	—	—	—	—	—	—	—	—	—	—	32	82.0	64.4	17.6	21.5

## 398 *Body Proportions of Different Breeds of Bacon Pigs*

A. *Length of side.* This was measured with a tape measure from the junction of the first rib with the sternum to the middle of the head of the femur in a straight line. This gives the most certain measure of the length of the side as it has two fixed bony points and there can be no doubt where the measurement begins and ends.

B. *Belly thickness.* This was measured with a pointed flat metal spike by insertion from the inner side until it meets the finger held against the skin opposite it. Measurements were always taken in the line of the nipples and between them. As the thickness may vary slightly in different parts of the belly, three measurements on each side were taken and the results averaged. These parts were: (1) one hand's breadth from the sternum; (2) at a point midway between the two—this last is the one used in the Bacon Pig Scheme; and (3) one hand's breadth from the ham end of the belly.

C. *Thickness of back fat.* This was measured in three different parts: (1) at the thickest part over the shoulder—this is usually at the level of the first rib; (2) at the thinnest part over the loin—this is usually at the level of the last rib; and (3) on the rump—as in this position the fat is very uneven, three measurements were taken and the results averaged. These measurements were taken with metal dividers, and the measurement was read off on a wooden ruler scale with a small slot at zero to hold one leg of the dividers.

D. *Flank thickness.* As the lower part of the belly in the line of the nipples is thicker than an area situated in the flank (about halfway from the top and bottom lines), marked by a patch of smooth white connective tissue on the inner side, a measurement was also taken here by the same method as employed in B.

A statistical analysis of all these measurements has been made to find out how the proportions change with breed, with increase in weight within a breed, with increase in length within a breed, etc. Since the weight of the side was known for each individual pig, whereas in many cases the live and carcass weight was only known for groups (all the pigs of one entry), the weight of the side of bacon has been used as the basis for calculation throughout. If it is required to know the relation of the live weight and the carcass weight to the measurements given, this can easily be calculated from the table which gives the corresponding live, carcass and bacon weights (Table I).

THE SHRINKAGE FROM LIVE WEIGHT TO CARCASS  
AND BACON WEIGHT

If it is desired to produce sides of bacon of a definite weight (say 55 lb.) one requires to know at what live weight the pig should be killed to produce sides of this weight. Moreover, one wants to know whether there is any difference due to breed or type in this respect. The data have therefore been analysed to show what weight of live pig and what weight of carcass it takes to produce bacon sides of different weights in the different breeds and crosses (Table I).

The live weight recorded here is that taken at the factory: what it would be on the farm would depend on the distance from the factory, mode of transport, etc. The live weight, and so carcass percentage, will vary too with the conditions of feeding, etc., as the figures given by Whetham<sup>(20)</sup> for pigs under different conditions show. For example, at 200 lb. live weight, the carcass percentage works out at 80.8 per cent for all pigs shown at the Smithfield Fat Stock Show, but only 72.5 per cent for pigs from the East Anglian Pig Recording Society<sup>(4)</sup> under commercial conditions. Our figures for the Dairy Show come intermediate between these—78.5 per cent—as they are not on the whole so fat as Smithfield Show pigs and, being picked specimens, come above those of the ordinary commercial animal.

From the averages of all breeds and crosses given in Table I and in Fig. 1 can be read off the live and carcass weight required to produce sides of bacon of weights ranging from 37 to 84 lb. For the weights of bacon sides most suited to market requirements—groups 55–59 and 60–64 lb. (see p. 404)—this is 198 and 209 lb. live weight and 155 and 164 lb. carcass weight respectively. It will also be seen that the later maturing breeds, which put on little fat, require especially at the heavier weights a little more live weight to produce a given weight of bacon than do early maturing types such as the Berkshire.

In calculating the shrinkage from live weight to carcass and bacon weights, the weights of the latter have been shown as a percentage of the live weight (Table I and Fig. 2). From these it will be seen that the actual live weight of the pig affects the percentage of bacon it will yield much more than does breed or type. As the bacon weight and live weight increase, so the bacon percentage increases from 53.2 per cent for a pig of 139 lb. live weight to 64.4 per cent for a pig of 261 lb. live weight, i.e. by 11.2 per cent. During this increase in live weight the carcass percentage also increases from 75.5 to 82.0 per cent (6.5 per cent); that is,

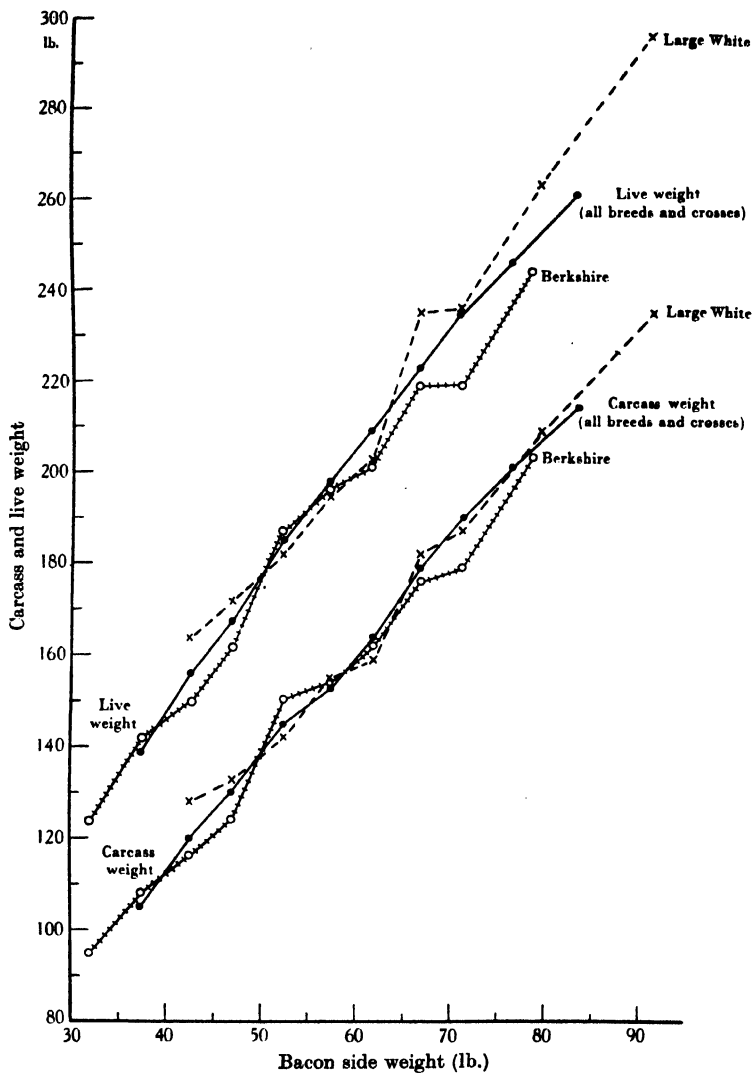


Fig. 1. Increase in live and carcass weight with increase in weight of bacon side.

one of the reasons why the bacon percentage increases with increase in the live weight is that the proportion of the offals (pluck, stomach, intestines, etc.) become less as the pig increases in weight (see Hammond (6)). This is not the only reason, however, why the bacon percentage

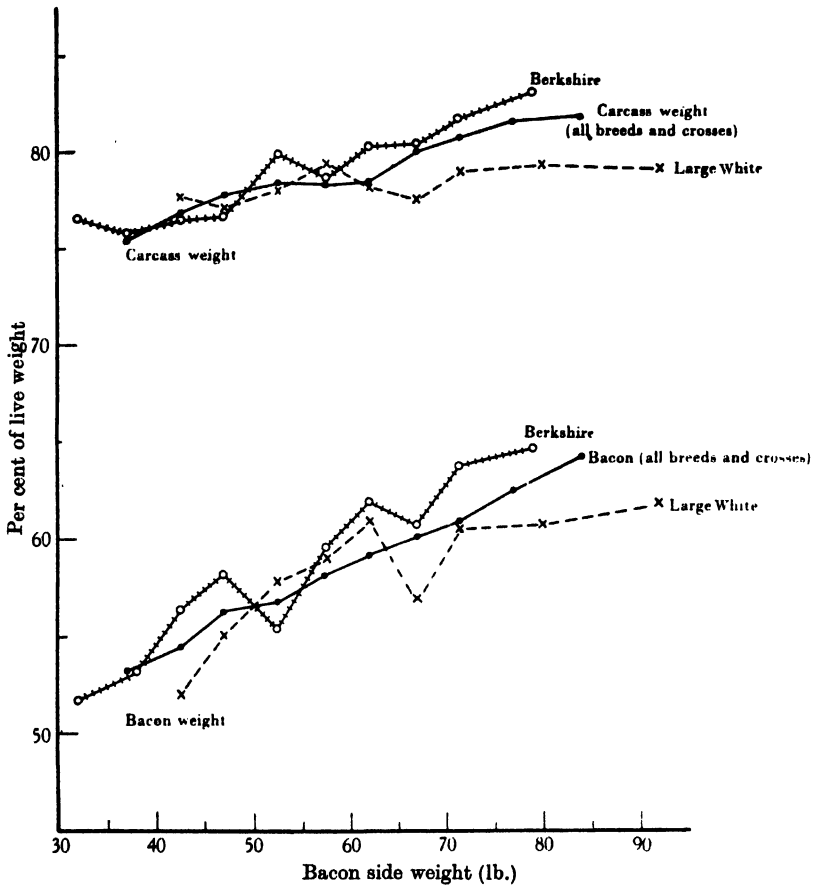


Fig. 2. Changes of carcass and bacon percentages with increase in weight of bacon side.

increases with increase in the live weight, for the differences between the carcass percentage and the bacon percentage (see last column in Table I) show a decrease from 22.3 per cent at 139 lb. live weight to 17.6 per cent at 261 lb. live weight (4.7 per cent), that is, in a small pig there is a higher proportion of head to take off and bone, etc., to trim out, in addition to any loss there may be in pickling and smoking.

## 402 *Body Proportions of Different Breeds of Bacon Pigs*

Since the demands of the trade are for 55–64 lb. sides, however, it would follow that there is an optimum carcass percentage and an optimum bacon percentage, rather than that the highest are the best. The highest carcass and bacon percentages are obtained at higher weights than these (55–64 lb.) and, as will be shown below, at these heavy weights the carcass becomes too fat to meet market requirements. The primary cause for the carcass percentage and bacon percentage increasing with the weight of the pig is (as is shown below) that, at the heavier weights, the fat on the carcass is increasing at a greater rate than the other parts of the body, such as the intestines, head, bones, etc., which are trimmed off before it is made into bacon. The optimum carcass percentage for an average bacon pig of about 200 lb. live weight, weighed at the factory before slaughter, would appear to be about 79 per cent, and the optimum bacon percentage about 58 per cent, for at this level the proportion of bone would be below pigs of the lower percentages, and yet the fat would not have developed to such large proportions as occur at the higher carcass and bacon percentages. Since the changes in the carcass percentage and bacon percentage are due to changes in the proportions of the tissues (offal, bone, muscle, fat) in the body, there is an optimum at about 79 per cent carcass and 58 per cent bacon where the fat, muscle and bone in the carcass will be in the proportions required by the consumer. Below this level the proportion of bone will be too large, and above this level the proportion of fat will be too high.

While the live weight is the main factor which affects carcass and bacon percentages, there is some variation in them due to breed and type. For example (see Table I and Fig. 2), whereas variation in live weight from 139 to 261 lb. causes a variation of 11·2 per cent in bacon percentage, the maximum average difference due to breed type (at 67–80 lb. bacon side weight) is only 3·6 per cent between the later maturing bacon type (Large White, average 59·5 per cent bacon) and the earlier maturing pork type (Berkshire, average 63·1 per cent bacon; and Middle White). From Fig. 2 it will be seen that the difference between these two types—bacon (Large White) and pork (Berkshire)—increases with increase in weight of the side, i.e. just as the carcass and bacon percentages go up with increase in live weight in all breeds and crosses, so it goes up more for a given increase in weight in the early maturing pork type such as the Berkshire than it does in the later maturing bacon type such as the Large White. As will be shown below, the bacon percentage goes up for the same reason—increase of fat in the carcass—whether this increase is due to increase in weight or to type differences. The first crosses between

the two types—pork and bacon—tends to be intermediate in both carcass and bacon percentage (see Table I).

If, as is suggested (see below), the changes in the carcass and bacon percentages are a mirror of the rate at which fat is added to the carcass, the breeds can be arranged in a definite order of maturity. Those which are late maturing will not put on much fat at the heavier weights, while those which are early maturing will add much. This order in increasing rate of early maturity (see Table I) would appear to be somewhat as follows:—Large White, Wessex, Essex, Gloucester Old Spot, Long White Lop Eared, Large Black, Berkshire and Middle White—although, with some of the breeds, a larger number of animals would be required before the exact order could be definitely decided.

Table II. *Shrinkage from carcass weight to bacon weight (shown as a percentage of the live weight)*

Breed	In bacon side weight (lb.)				Average
	50-54	55-59	60-64	65-69	
Middle White	19.0	19.7	(18.4)*	15.9	18.2
Berkshire	(19.5)*	19.1	18.4	19.8	19.2
Large White	20.3	20.5	17.4	20.3	19.7
Long White Lop Eared	20.3	20.2	20.0	19.2	19.9
Large Black	22.9	20.8	19.4	21.3	21.1
Gloucester Old Spots	23.8	22.3	20.5	20.7	21.3
Wessex	24.7	21.5	18.9	21.5	21.6
Average all breeds	21.5	20.6	19.0	19.8	—

\* Figures in brackets interpolated for purposes of averages.

As has been pointed out above, the difference between the carcass percentage and the bacon percentage is probably a measure of the shrinkage due to the removal of the head and to trimming out of the bone; this is higher in pigs of low weight than in those of high weight. If the various breeds are compared in this respect, it will be seen that they come in the order shown in Table II. This order may probably be taken as an order of ascending coarseness of bone. The Middle White, Berkshire and Large White, being fine in the bone, will have less weight to trim off the carcass than will breeds such as the Gloucester Old Spots or Wessex. Again, however, the exact order of the breeds in this respect requires confirmation from larger numbers of individuals. Within any one breed there is of course a considerable variation in this respect according to type.



## 404 *Body Proportions of Different Breeds of Bacon Pigs*

### MARKET REQUIREMENTS: OPTIMUM PROPORTIONS

While the market requirements will vary slightly according to different districts and classes of trade, there have been definite changes taking place in Wiltshire sides (with which this paper deals) towards a carcass which contains less fat than formerly.

At the 1927 Dairy Show the Ministry of Agriculture obtained "ideal" sides of bacon picked out by the Provision Trades Associations and Exchanges of Liverpool, Bristol, London, Glasgow and Manchester (16). These were measured in the same way as the other sides, and the data obtained will illustrate the ideal carcass proportions which the British bacon distributors at that time desired. The weights and measurements of these sides are given in Table III. The weights fall into two of our weight classes—55–59 lb. and 60–64 lb.—and, as would be expected, there is a slight increase in all measurements, with the exception of the flank, from the lower weight group to the higher.

Since that time the Pigs' and Bacon Marketing Boards have come into existence, and they have decided on certain standards on which to base payments for bacon pigs. These have varied from time to time and specify some only, and not all, of the measurements of the carcass which we have taken. The standards so fixed for the year 1934–5 are given in Table III.

Table III. *Measurements of bacon sides suited to market requirements*

(a) Selected by Provision Trades Associations, 1927  
Average measurements (mm.)

Bacon side weight lb.	No. of sides	Average measurements (mm.)					
		Length	Back fat (av. 5 measurements)	Loin fat	Shoulder fat	Belly thickness	Flank thickness
55–59	5	792	38	30	46	39	29
60–64	7	795	44	35	51	43	28

(b) Pigs' Marketing Board Standards, 1934–5\*

Class	Grade	Maximum Minimum				
(1) 140–170 lb. carcass weight	C	—	—	—	51	29
	B	—	—	—	45	or 38
	A	—	—	—	45	and 38

(c) Danish Litter-testing Station averages, 1933–4\*

Bacon side weight lb.	No. of sides					
60	264	910†	36	—	—	33

\* These relate to the fresh carcasses and not to the cured bacon.

† This measurement includes neck lengths and so is not comparable with the lengths given in this paper.

As the Danes have for long specialized in producing Wiltshire sides for the British market, and since their bacon commands the best price on the English market, we have also given in Table III the average figures for all pigs passing through the litter-testing stations in Denmark for the year 1933-4, as given by Clausen (3). Unfortunately the measurement for length of side was taken in a different way, and so is not comparable with ours.

It is with such bacon standards that the proportions of the sides of different types of pigs are compared below. These two last standards refer to measurements on fresh carcasses and not on the cured bacon as ours do, but although no large number of figures for the difference between the two have been obtained, it is believed that the difference is comparatively small.

The reasons why such weights and proportions in bacon sides are required may be stated briefly, for they have a scientific basis although they have been arrived at by a system of trial and error.

*The weight of the side* is very much above that shown in Table III will generally be too fat, for it is fat which accounts for most of the weight put on in the later stages of growth. If the weight of the side is very much less on the other hand, the actual thickness of the streak (belly) is liable to be too small, so that the rashers dry up on cooking; moreover, there will usually be a high proportion of bone.

Unless the side is of a *certain length* at these weights it will be too fat, and too much of the weight will be due to depth of the side, i.e. to development of the belly and lower ribs (cheap parts) instead of the development along the back which contains the valuable thick blocky muscles of the body (9). By requiring a certain length of side for a given weight it is then assured that the depth of the body will be limited.

Although, as has been stated above, one does not require a great depth of belly because of the low price it fetches as compared with the back, yet *the belly* must have a certain thickness before it is saleable, because of the drying up on cooking. As thick a belly as possible is therefore desired when the conditions of length have been fulfilled: to obtain this a pig with short well-sprung ribs rather than a deep flat-sided one is required. As will be shown below, while it is easy to get thick bellies by overfattening the pig (shown by the relation of belly thickness to back fat thickness), the trade requires a thick belly which is produced by thickness of muscle rather than by fattening. In this way the arbitrary measurements for *back fat* (which should be as small as possible) and belly thickness (which should be as large as possible)

## 406 *Body Proportions of Different Breeds of Bacon Pigs*

adopted by the Pig and Bacon Boards have a scientific basis in that, by the reduction of the thickness of back fat to as low a measure as possible, and at the same time having as great a thickness of belly as possible, the chances of having a side with a high proportion of muscle and low proportion of fat are increased. In fact, this high proportion of muscle and low proportion of fat is what is really being aimed at by fixing such arbitrary standards of measurements, for it has been learned by experience that in the pig such proportions of the body are associated with lean sides.

### THE CHANGES IN THE PROPORTIONS OF THE SIDE WITH INCREASE IN WEIGHT

#### (a) *General*

The changes in the measurements of the different parts as the weight of the side increases are shown for all breeds and crosses considered together in Table IV.

From the range of weights within which commercial bacon production lies (between broken lines in Table IV) and within which most of the exhibits fall (sides of 47–72 lb. which correspond to fresh carcass weights of about 130–190 lb.), the rate of increase has been calculated for each measurement. These rates of increase, calculated for each 5 lb. increase in weight of cured side and also for each 10 lb. increase of fresh carcass, are shown at the base of Table IV. Thus for each 10 lb. increase in the weight of the fresh carcass, the length of the bacon side increases by 12.3 mm., the thickness of the fat over the loin by 2.6 mm., fat over the shoulder 2.4 mm. and thickness of belly by 1.7 mm.

On the basis of these natural changes in the proportions, it is possible to make comparisons between carcasses of different weights and, by taking measurements at one weight, to make a rough estimate of what the measurements would have been at another weight. These natural changes in the proportions of the carcass with increase in weight, however, do not correspond with the market requirements at the different weights, for with increase in weight of side there is a natural tendency for additions of fat to be added in excess of market requirements, especially in some breeds (see p. 414). For example, while the market requirement for 60–64 lb. sides is 45 mm., or under, of back fat at the shoulder (see Table III), the actual average of all the sides shown at this weight is 56.8 mm. (see Table IV).

Table IV. *Changes in the measurements of the sides with increase in the weight of the side (all breeds and crosses)*

Bacon side weight lb.	No. of sides	Side length mm.	Belly thick- ness mm.	Back-fat thickness (mm.)						Av. 5 measure- ments	Flank thick- ness mm.
				Shoulder	Loin	Rump					
						1	2	3			
30-34	2	688	24.0	38.0	17.0	23.0	19.0	29.0	25.0	15.0	
35-39	15	697	29.4	44.3	21.0	29.2	21.4	32.8	30.2	18.6	
40-44	25	732	29.7	45.8	24.4	33.4	25.8	38.8	34.0	19.8	
45-49	50	752	31.8	49.4	27.2	37.8	30.3	41.1	37.2	20.0	
50-54	195	787	33.6	51.4	29.7	40.7	32.9	43.4	39.5	20.4	
55-59	240	801	35.3	54.2	31.9	43.0	34.5	45.8	42.0	21.8	
60-64	145	808	37.2	56.8	34.7	46.3	36.7	48.0	44.7	23.5	
65-69	51	813	41.4	63.0	41.8	52.2	44.0	56.6	51.4	26.9	
70-74	43	826	41.9	64.0	41.1	55.3	39.3	56.3	51.3	25.9	
75-79	21	814	42.4	65.7	43.7	55.3	39.6	54.0	51.6	—	
80-84	24	833	43.8	65.2	46.7	58.9	47.3	57.1	54.9	—	
85-89	7	876	44.0	64.9	49.0	61.7	47.1	62.3	55.7	—	
90-94	5	898	43.0	68.0	40.0	56.0	41.0	60.0	53.0	—	

*Increase from 47-72 lb. bacon side (130-190 lb. carcass weight)*

	Side length mm.	Belly thickness mm.	Back-fat thickness (mm.)				Av. 5 measurements	Flank thickness mm.
			Shoulder	Loin	Rump			
For 25 lb. increase in bacon weight	73.7	10.1	14.6	15.9	13.9		14.1	5.9
For each 5 lb. increase in bacon weight	14.74	2.02	2.92	3.18	2.78		2.82	1.18
For each 10 lb. increase in carcass weight	12.28	1.68	2.43	2.65	2.32		2.35	0.98

Scales of marks and measurements for defining the optimum measurements at different carcass weights have been published by Davidson *et al.* (5), so this question will not be dealt with here. These tables for judging bacon carcasses are based on:

(1) An optimum measurement for the thickness of fat at the loin, which should not be above or below 21 mm. for fresh carcasses of 150-59 lb. The shoulder back fat should be as thin as possible and should not exceed 45 mm. at this weight, although it may increase slightly as the weight of the carcass rises.

(2) The minimum requirements for length of side increase with the weight of the carcass and, other things being equal, the longer they are for their weight the better. 830 mm. for a carcass of 150 lb. will give a side of very good proportions.

(3) The belly should be as thick as possible and the thickness required increases with the weight of the side. Since the composition as well as

## 408 *Body Proportions of Different Breeds of Bacon Pigs*

the actual thickness of the streak is important, in competitions it is advisable to cut it and judge by eye against a photographic scale.

In order to show how the conformation of bacon sides of different weights varies from the average proportions of a bacon side of 57 lb., the weights and measurements of the different parts have been shown in each case as a percentage of the weight or measurement respectively at 57 lb. weight (see Table V and Figs. 3 and 4 in which some of the irregularities of the figures have been smoothed). It will be seen that the rate of increase is not proportional in all the parts. As might be expected at this comparatively late stage of growth (see diagrams and photographs (7)) the proportional increase in bacon weight is very much greater than the proportional increase in side length, i.e. side length growth develops comparatively early in life. Intermediate between these two come belly thickness and shoulder fat; both increase in proportions at a faster rate than side length, but neither increases so fast as side weight (see Fig. 3). Over these ranges of side weights the only one part, of those measured, which is increasing at the same rate as, or slightly faster than, bacon weight, is that of the thickness of the fat over the loin (see Fig. 4), i.e. this part, like bacon weight, is a late maturing part of the body.

Table V. *Changes in the proportions of the side with increase in weight of side (all breeds and crosses)*

Each weight and measurement is shown as a percentage of its weight or measurement in 55-59 lb. sides.

Bacon side weight lb.	Side weight lb.	Side length mm.	Belly thickness mm.	Back-fat thickness (mm.)			Flank thickness mm.
				Shoulder	Loin	Rump (3)	
30-34	56.2	85.8	68.0	70.1	53.3	57.6	68.8
35-39	65.0	86.9	83.3	81.7	65.8	67.6	85.3
40-44	73.7	91.3	84.1	84.5	76.5	79.5	90.8
45-49	82.5	93.9	90.1	91.1	85.3	88.6	91.7
50-54	91.2	98.2	95.2	94.8	93.1	94.9	93.6
55-59	100.0	100.0	100.0	100.0	100.0	100.0	100.0
60-64	108.8	100.8	105.4	104.8	108.8	108.2	107.8
65-69	117.5	101.4	117.3	116.2	131.0	123.9	123.4
70-74	126.3	103.1	118.7	118.1	128.8	122.4	118.8
75-79	135.0	101.5	120.1	121.2	137.0	120.8	—
80-84	143.8	103.9	124.1	120.3	146.4	132.4	—
85-89	152.5	109.3	124.6	119.7	153.6	138.8	—
90-94	161.4	112.1	121.8	125.5	125.4	127.3	—

It is interesting to compare the rates of increase occurring in the thickness of the subcutaneous layer of fat in the different regions of the body (Fig. 4). In the region of the shoulder it matures earliest, in the region of the rump next, and in the region of the loin (about the level of

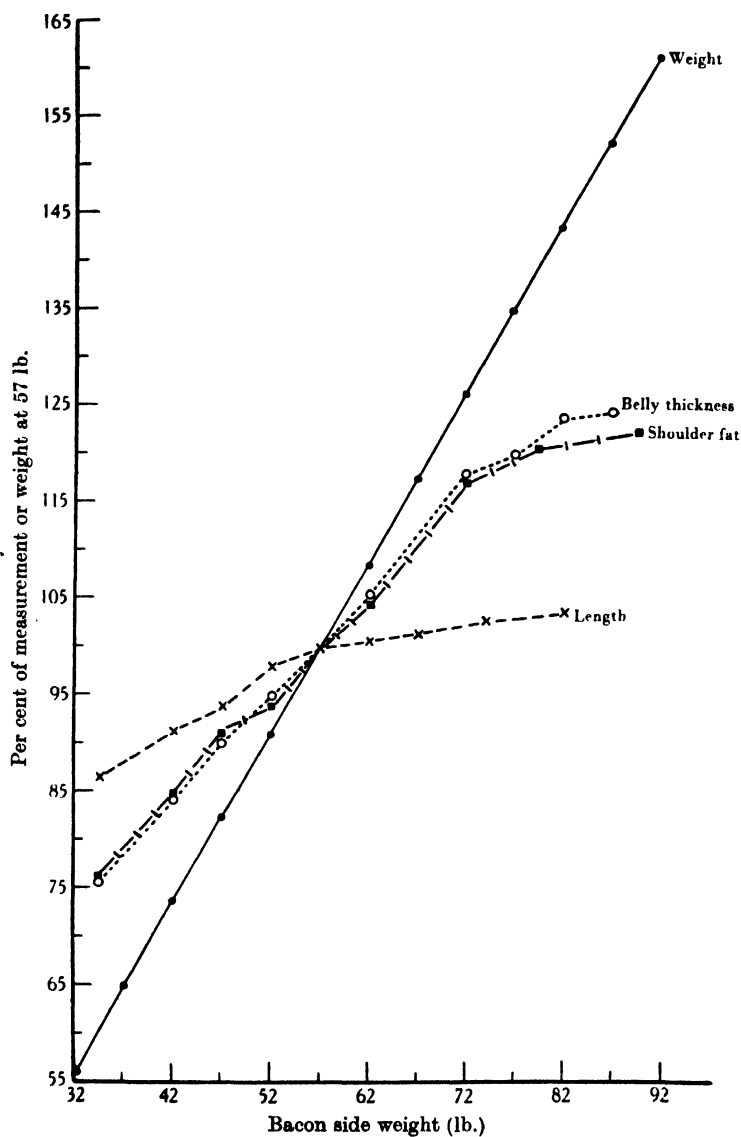


Fig. 3. Changes in the proportions of the sides with increase in the weight of the side.

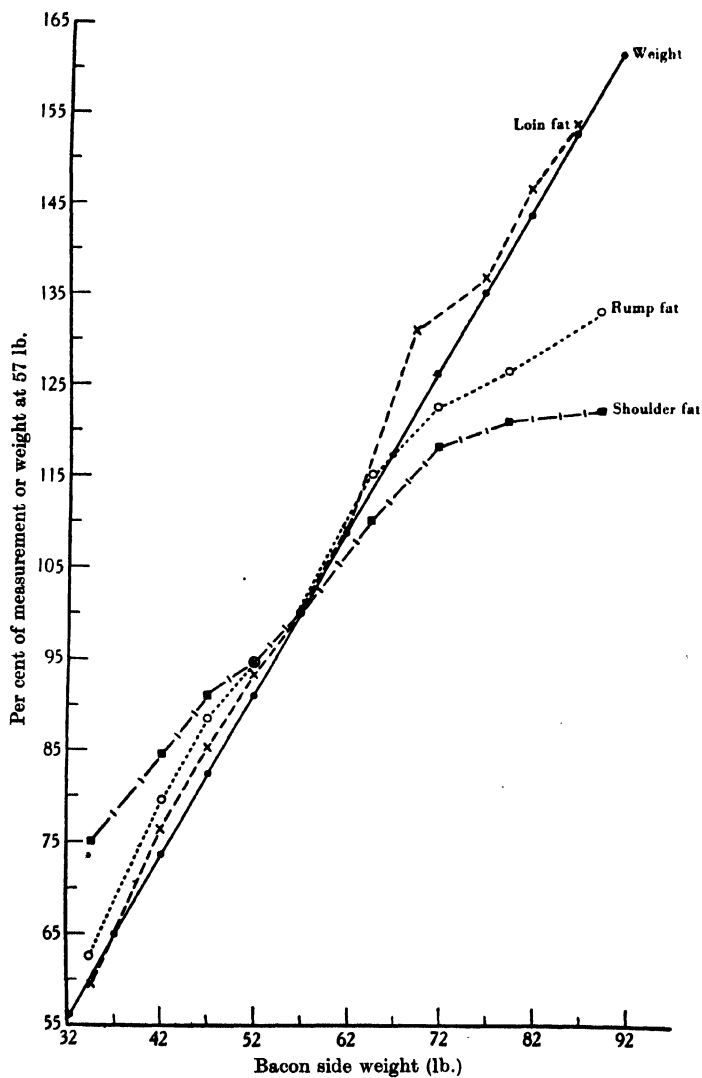


Fig. 4. Changes in the proportions of the fat in the sides with increase in the weight of the side.

the last rib) last. In the light side the fat on the shoulder is proportionally large and that on the loin proportionally small, the rump area being intermediate, but as the side increases in weight the increase made on the loin is proportionally greater than that made on the shoulder (see Plate VI). This fact is illustrated in another way (see Table VI and Fig. 5) by showing the thickness of the loin fat and rump fat at each weight as a percentage of the thickness of the shoulder fat at that weight. As the weight of the side increases from 33 to 87 lb., the loin fat increases from 44.7 to 75.5 per cent (30.8 per cent) and the rump fat from 62.4 to 87.8 per cent (25.4 per cent) of the thickness of the shoulder fat at these weights.

Table VI. *Changes in the back fat with increase in bacon side weight (all breeds and crosses)*

Bacon side weight lb.	Back-fat thickness (mm.)			Proportional to shoulder fat (%)		Rate of increase (mm. per 5 lb. bacon weight)		
	Shoulder	Loin	Rump	Loin	Rump	Shoulder	Loin	Rump
30-34	38.0	17.0	23.7	44.7	62.4	3.35	3.17	3.82
35-39	44.3	21.0	27.8	47.4	62.8			
40-44	45.8	24.4	32.7	53.3	71.4			
45-49	49.4	27.2	36.4	55.1	73.7			
50-54	51.4	29.7	39.0	57.8	75.9			
55-59	54.2	31.9	41.1	58.9	75.8	3.15	2.85	2.82
60-64	56.8	34.7	43.7	61.1	76.9			
65-69	63.0	41.8	50.9	66.3	79.2			
70-74	64.0	41.1	50.3	64.2	78.6			
75-79	65.7	43.7	49.6	66.5	75.5	0.30	2.63	2.23
80-84	65.2	46.7	54.1	71.6	83.0			
85-89	64.9	49.0	57.0	75.5	87.8			
90-94	68.0	40.0	52.3	58.8	78.4			
Average rate of increase						2.45	2.87	3.03

The actual rate of increase of the thickness of the back fat slows down as the sides increase in weight; for example, in the rump fat the rate of increase falls from 3.82 mm. per 5 lb. side increase at 30-54 lb., to 2.82 mm. at 55-74 lb. and to 2.23 mm. at 75-94 lb. (see Table VI). The rate of falling off, however, varies in the different regions of the back. The slackening of the rate of growth is naturally greatest in the most early maturing part—the shoulder. The rate of growth in the shoulder falls from 3.35 mm. at 30-54 lb. to 0.30 mm. at 75-94 lb. (a drop of 3.05 mm. in growth rate), whereas that of the rump falls from 3.82 to 2.23 mm. (a drop of 1.59 mm.), and that of the loin from 3.17 to 2.63 mm. (a drop of 0.54 mm.).

This is the scientific explanation of the item in the scale of points which is now used at the Dairy Show and the N.P.B.A. Show which



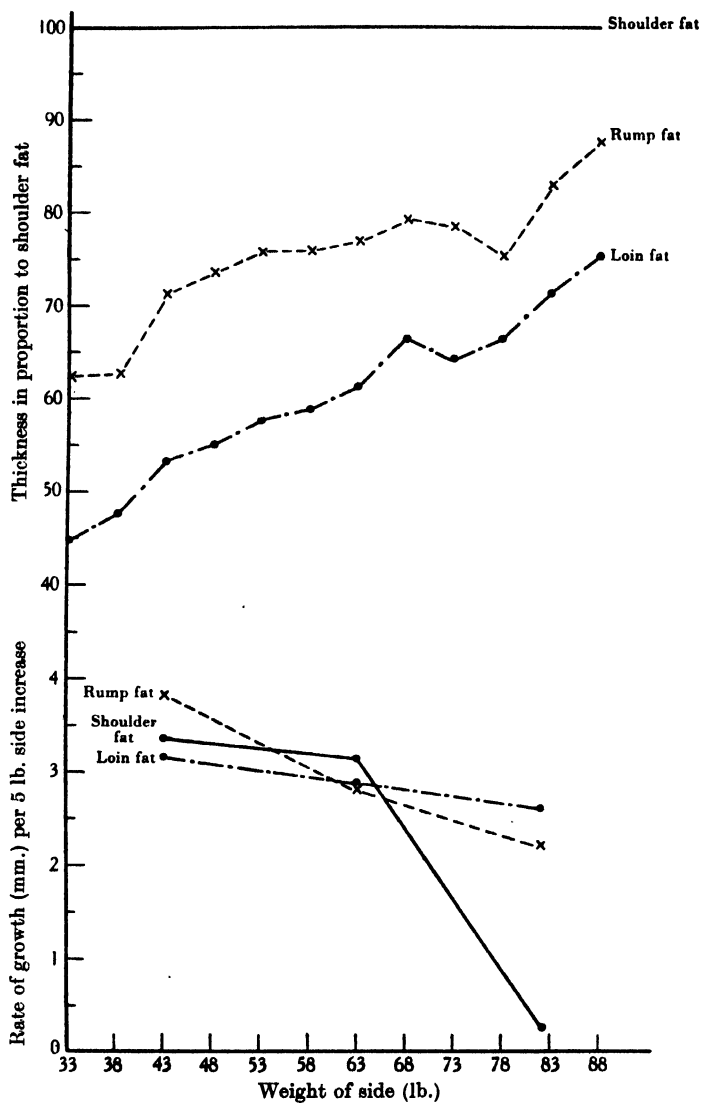


Fig. 5. The back fat (all breeds).

reads: "Reduction of back fat from shoulder to gammon—5 points", for this implies that the full fat deposits have not yet been developed, and so the side will cut with a lower proportion of fat than one in which the thickness at the shoulder and at the loin is more nearly equal. As will be shown below, breeds differ, according to their rate of maturity, in the rate (and so the actual side weight) at which the thickness of the loin fat begins to approach the thickness of the shoulder fat (see Plate VI).

From these measurements it would appear that in the pig, as has already been shown for the sheep and other animals<sup>(8)</sup>, the cervical and thoracic parts are the earliest maturing region of the vertebral column, the caudal and sacral area coming next, while the latest maturing area is the lumbar in the region of the last rib—the most valuable part<sup>(9)</sup>. Callow<sup>(2)</sup> has also demonstrated this in the back fat of the pig and, as confirmatory evidence also, Shaw<sup>(19)</sup> finds that the number of ribs in the pig is very variable and that the extra ribs which are added at the loin end can easily be selected for.

It is for this reason, in addition to the fact that it is the most valuable part, that the cut should be made through the loin at the level of the last rib when judging a carcass. If the latest maturing part of the body is well developed there is no need to look at the rest, although if an early maturing part, such as the shoulder, were cut there would be no guarantee that the later maturing parts of the body would be equally well developed. For this reason at many Shows the side is now cut through at the loin, and not at the shoulder as was formerly done.

If it is required to estimate the total amount of the fat in the carcass (as has been done by Hankins & Ellis<sup>(10)</sup>) from one measurement, it would appear that the thickness of the fat at the last rib would be the best measure to adopt for this purpose, at any rate during the later stages of growth.

Another fact shown in Table V is that with increase in weight of the side, the changes in the proportions of certain parts are closely related. For example, it has already been mentioned that, within this range of weights, the rate of increase of the thickness of the fat over the loin is much the same as that of the weight of the sides as a whole (see Fig. 4). The thickness of fat over the shoulder, however, follows closely that of belly thickness (see Fig. 3) and flank thickness (see Table V). The actual thickness and the actual rates of increase in thickness are very different (see Table IV), but over this range of bacon weights the increase in thickness of these three parts is proportional, so that the same relative conformation between them is maintained throughout this range of

## 414 *Body Proportions of Different Breeds of Bacon Pigs*

weights. Since the actual measurements of these three parts are very different at the lowest bacon weight measured, it is evident that the proportions of these three parts to one another is determined by different rates of growth at lower weights than those considered here. Since the thickness of belly will include some muscle growth in addition to fat, it might be expected to have an earlier development than the fat. The relationship between these two parts—shoulder back fat and belly thickness—is important, for the bacon curers require a side in which the back fat is thin but the belly thick. As over this range of weights the proportions alter together, no better proportions can be obtained by marketing the pigs at one weight rather than another. It is shown below (p. 419), however, that the solution of this problem lies in breed selection and in obtaining the rapid growth of the muscular parts of the belly in the early stages of life, for in the latter stages of life fat is added to both belly and shoulder in similar proportions.

### (b) *Breed differences*

While the above is a general statement of the changes which occur with increase in weight of the side, the different breeds vary considerably in the rate at which these changes occur. In the first place there is a difference in the distribution of the sides according to weight in the different breeds. The Middle White and Berkshire tend to be sent at lower weights than Gloucester Old Spots or Large White (see Table VII): this is probably because they attain bacon proportions at a lower weight. At the same weight of side some breeds give a much longer side than others; for example, at 55 lb. side weight the Large White average 817 mm. as compared with 786 mm. for the Essex and 753 mm. for the Middle White (see Table VIII). In average back-fat measurements, too, the breeds vary considerably at constant side weight (see Table IX); for example, at 55 lb. bacon side weight the fat thickness (average of five measurements) varies from 37 mm. in the Large White to 48 mm. in the Large Black. The rate at which fat is put on varies considerably in the different breeds—thus from a 40 lb. to a 60 lb. side it increases from 33 to 39 mm. (6 mm.) in the Large White, whereas for the same range it increases from 35 to 45 mm. (10 mm.) in the Berkshire. At the same weight of side, breeds vary too in the thickness of the belly (see Table X). In general, those with the thicker back fats have the thicker bellies; but this does not always apply, since the thickness of the belly may be due to thickness of lean meat as well as to additions of fat. For example, while at 55 lb. side weight the thickness of the back fat of the Large Black is

Table VII. *Distribution of bacon sides according to weight and length in the different breeds and crosses*

Bacon side sight lb.	No. of observations										
	Large White	Long White Lop Eared	Wessex	Gloucester Old Spots	Large Black	Essex	Berkshire	Middle White	Large White × Large Black	Large White × Berk- shire	Large White × Middle White
30-	—	—	—	—	—	—	2	—	—	—	—
35-	—	—	—	—	—	—	9	6	—	—	—
40-	7	—	—	—	—	—	14	4	—	—	—
45-	21	5	—	—	—	10	6	2	—	4	2
50-	78	22	8	13	27	—	2	9	6	7	8
55-	86	23	8	15	42	7	16	8	20	4	11
60-	33	5	27	25	14	8	11	2	10	6	2
65-	2	2	3	15	15	—	9	3	—	—	2
70-	8	6	4	16	4	—	9	—	—	—	—
75-	—	—	—	4	3	6	8	—	—	—	—
80-	3	—	—	9	4	4	—	—	—	—	—
85-	—	—	—	3	—	4	—	—	—	—	—
90-	5	—	—	—	—	—	—	—	—	—	—
Bacon side gth mm.											
-695	—	—	—	—	—	—	12	2	—	—	—
-715	—	—	—	—	—	10	6	6	—	—	—
-735	6	—	—	—	—	—	12	5	—	—	—
-755	5	5	—	—	5	—	7	13	—	—	8
-775	43	13	3	5	21	4	12	8	3	10	9
-795	55	24	16	19	33	8	20	—	11	6	—
-815	66	13	19	26	24	8	15	—	14	—	9
-835	48	8	6	24	13	3	—	—	12	4	2
-855	19	—	4	13	16	7	—	—	—	—	1
-875	11	—	—	14	—	5	—	—	—	—	—
-895	9	—	2	—	—	—	—	—	—	—	—



Table IX. Back-fat (average of five measurements) changes with increasing weight of side in different breeds and crosses

[illegible]



greater than that of the Berkshire (48–43 mm.), the thickness of the belly is less (36–38 mm.). At the bottom of Table XVII is given the ratio of thickness of belly (=1) to thickness of back fat in different breeds. The lower this ratio is the thicker should be the lean meat in proportion to the fat, and, failing direct measurement of the lean meat in a carcass, this would appear to be one of the best indicators of it. In this respect breeds come in the following order of decreasing thickness of lean in proportion to fat—Berkshire, Large White, Essex, Middle White, Wessex, Gloucester Old Spots, Large White Lop Eared, and Large Black. Unlike other points considered here, these ratios have not been taken in relation to a constant weight of side but are averages of all sides shown.

These breed differences in the proportions of the thickness of the back fat at any one weight make it appear probable that in the limitation of the ration, suggested by Mansfield & Trehane<sup>(13)</sup>, for reducing the fat put on by bacon pigs during the latter part of the feeding period, the time and extent of the limitation of feed necessary to obtain the optimum results will depend on the rate of maturity of the breed, or type within the breed. Those breeds which tend to put on more fat at any one weight will have to be limited in their ration more and earlier than will those which put on less fat at that weight (see Table IX).

Table XI. *Breed differences in the maturity of the back fat*

Bacon side weight lb.	Ratio— $\frac{\text{shoulder fat (mm.)}}{\text{loin fat (mm.)}}$	
	Berkshire	Large White
40–49	1.76	1.95
50–59	1.80	1.86
60–69	1.62	1.85
70–79	1.60	1.81

As has been shown above, the depth of fat on the loin matures later than the depth of fat on the shoulder, and so the ratio between the two diminishes as the side increases in weight (see Fig. 5). As might be expected, in the early-maturing breeds such as the Berkshire, these changes in ratio between the thickness of fat in the two areas occur at lower weights than in the late-maturing breeds such as the Large White. Thus, at any one bacon side weight, there is a larger difference between shoulder- and loin-fat measurements in the Large White than in the Berkshire (see Table XI), this taper from shoulder to loin indicating a carcass which is not as yet fully mature and has not yet attained its maximum fat development (see also Plate VI).



## 420 *Body Proportions of Different Breeds of Bacon Pigs*

Table XII. *Measurements of cross-bred and pure-bred pigs compared at similar bacon side weights and lengths*

Breed and cross	No. of weight groups	Side weight lb.	Side length mm.	Back-fat thickness (5 measurements) mm.	Belly thickness mm.
(a) At similar bacon side weights					
Large White	3	—	808	37	34
Large Black	3	—	794	50	36
Mean of parent breeds	—	—	801	43	35
Cross-bred	3	—	803	39	35
Large White	4	—	802	36	33
Berkshire	4	—	751	40	36
Mean of parent breeds	—	—	776	38	34
Cross-bred	4	—	783	38	35
Large White	5	—	810	36	33
Middle White	5	—	758	46	36
Mean of parent breeds	—	—	784	41	34
Cross-bred	5	—	790	40	36
(b) At similar bacon side lengths					
Large White	3	56	—	36	33
Large Black	3	62	—	52	38
Mean of parent breeds	—	59	—	44	35
Cross-bred	3	58	—	39	36
Large White	3	54	—	36	32
Berkshire	3	65	—	44	40
Mean of parent breeds	—	59	—	40	36
Cross-bred	3	57	—	39	36
Large White	2	51	—	35	31
Middle White	2	57	—	40	36
Mean of parent breeds	—	54	—	37	33
Cross-bred	2	52	—	38	34

When breeds are crossed and the pure parent breeds are compared with the cross-bred at similar body weights (see Table XII), there is an indication that in body length the cross-breds are slightly above the mean of the parent breeds, while in back-fat thickness and in belly thickness they are practically intermediate. The numbers of individuals on which these conclusions are based, however, are too few to make these conclusions absolutely certain, and experiments are needed to verify these observations. Schmidt *et al.* (18), however, found in various crosses between German breeds that, when compared at equal live weights, the crosses had a back fat equal to that of the parent breed with the higher value.

### THE CHANGES IN PROPORTIONS WITH INCREASE IN LENGTH

Since, as has been shown in the previous section, the length of the side is the earliest maturing part measured, this has been taken as a basis on which to compare the changes in the proportions of the body as the pig

grows up and as between one breed and another. It is a measure of bone development, whereas the other measurements are of the later-developing fat (back fat) or muscle and fat together (belly).

How the various parts change in their proportions as the pig increases in length of body will first be considered within one breed—the Large White, which is of bacon type and is represented in largest numbers. These changes will then be compared with those occurring in another breed—the Berkshire, which is of pork type.

Table XIII. *Changes in weights and measurements as the side increases in length within a breed*

Bacon side lengths mm.	No.	Live lb.	Carcass lb.	Bacon side lb.	Belly thick- ness mm.	Back-fat thickness (mm.)			Flank thick- ness mm.
						Shoulder	Loin	Rump	
(a) Large White									
720-	6	159	124	43	29	46	25	35	22
740-	5	178	139	51	31	51	25	36	22
760-	43	183	143	52	32	48	26	35	21
780-	55	179	145	54	32	51	26	36	22
800-	66	190	148	56	33	50	26	37	22
820-	48	196	154	58	35	50	26	36	21
840-	19	212	164	65	35	51	28	39	21
860-	11	221	172	64	35	49	27	37	21
880-	9	267	211	81	39	64	37	50	—
(b) Berkshire									
680-	12	140	107	38	27	46	24	33	19
700-	6	143	109	42	29	45	29	35	20
720-	12	174	136	52	36	52	29	39	22
740-	7	175	137	52	36	51	28	38	24
760-	12	193	154	61	39	58	33	43	—
780	13	208	167	66	40	59	36	43	23
800-	11	219	177	69	42	65	39	45	27

As the Large White increases in side length the weight of the bacon side increases in both actual weight (Table XIII) and in weight in proportion to length (Fig. 6). The belly thickens and the loin back-fat thickness also increases somewhat in actual measurement, but only very slightly in proportion to length. The shoulder back fat, however, although it increases very slightly in actual measurement, decreases in proportion to length as the length of the side increases.

In an early-maturing pork type such as the Berkshire, however, a very different state of things exists; not only is the actual increase in all measurements marked (Table XIII), but the proportional development in relation to length also increases in each part (Fig. 6). For each unit of body-length increase, the bacon side weight increases much more in the Berkshire than in the Large White.

## 422 *Body Proportions of Different Breeds of Bacon Pigs*

On account of space it is not possible to give here similar details for all the breeds, but the two types having been dealt with in detail above, the smoothed curves (Fig. 7) showing the relations of bacon side weight

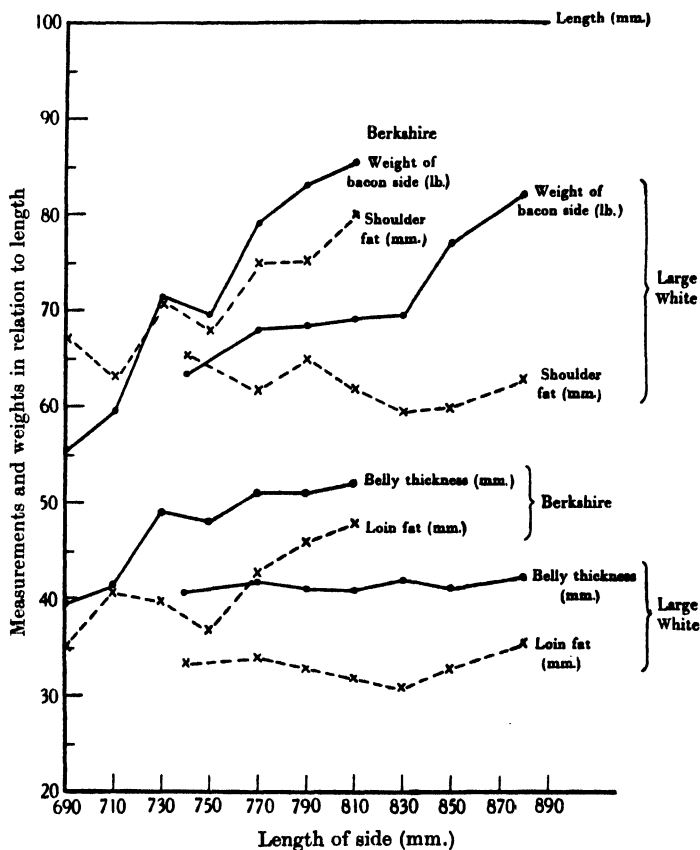


Fig. 6. Proportions in relation to length with increase in length of side (Large White and Berkshire).

to length with variations in length of the side, will serve to illustrate the main differences between the breeds.

The figures in Table XIII show what takes place as the pig grows up, for length increases with age and live weight. They do not necessarily show, however, what changes occur in the proportions of the side as it is lengthened at any one age and bacon weight, except in so far as the Large White has longer sides at the same bacon weight than has the Berkshire.

For example, at a side length of 780-799 mm., the Large White has a bacon side weight of 54 lb. as compared with 66 lb. for the Berkshire,

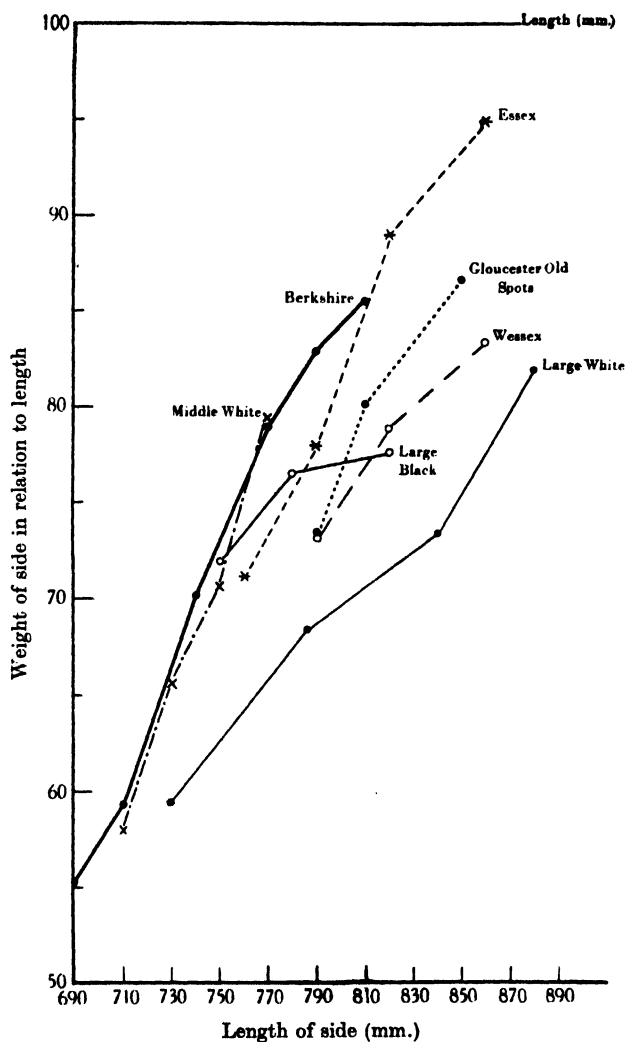


Fig. 7. Proportions of bacon side weight in relation to length (=100) with increase in length of side in different breeds.

and a loin back-fat measurement of 26 mm. as compared with 36 mm. for the Berkshire. In order to show what changes occur within a breed when the pig is lengthened at any one weight, Table XIV has been

## 424 *Body Proportions of Different Breeds of Bacon Pigs*

calculated for the Large White breed and for the average of eight sets of sides in five different breeds. These two sets of figures confirm one another and show that as the side is lengthened at any one weight so the thickness of the back fat is decreased, while there is no constant effect on the thickness of the belly. In other words, the thickness of the back fat can be decreased at any bacon weight by lengthening the side at that weight, and this can be done without affecting the thickness of the belly. The distribution of the individuals of different breeds according to the side length is shown in Table VII. There is considerable variation within each breed and no doubt, if it were desired by the breeders, the breeds now considered to be too short could be lengthened to conform to bacon type.

Table XIV. *Effect of increasing the length of the side at a constant side weight within a breed*

Breed	Side length (mm.)		Side weight lb.	Belly thickness mm.	Back-fat thickness (mm.)			Flank thickness mm.
					Shoulder	Loin	Rump	
Large White (All at 55 to 64 lb. side weight)	790	Short	59	33.5	53.7	28.0	38.3	23.3
	821	Medium	60	34.8	51.1	27.5	35.9	22.4
	858	Long	61	32.5	50.9	27.8	37.2	22.0
Eight sets in five different breeds	771	Short	58	35.2	56.8	34.1	42.9	22.6
	800	Medium	57	35.3	55.4	33.0	42.6	22.0
	831	Long	58	35.2	54.7	32.3	43.4	23.4

As might be expected from the general conformation and differences in the rate of maturity of the breeds, the weight of a side of any given length varies considerably in the different breeds. As far as can be judged from the data available (Table XV), the breeds come in the following order of increasing weight for constant length of side—Large White, Long White Lop Eared, Wessex, Gloucester Old Spots, Large Black, Essex, Berkshire, and Middle White.

For any given length of side too, the thickness of the back fat varies in the different breeds (see Table XVI): the difference between the Large White, with a small amount of back fat per unit of length, and the Large Black and Gloucester Old Spots, with a large amount, is very marked.

In belly thickness per unit of length (Table XVII), on the other hand, the Large White has the smallest, in fact this is the weakest point of this breed from a bacon point of view. Other breeds come in the following order of increasing thickness of belly—Long White Lop Eared, Wessex, Gloucester Old Spots, Large Black, Essex, Middle White, Berkshire. It will be noticed that this is the same order in which breeds stand as regards weight for a constant length of side.

Table XV. *Bacon side weight changes with increasing length of side in different breeds and crosses*

Bacon side length (mm.)	Bacon side weight (lb.)										
	Large White	Long White Lop Eared	Wessex	Gloucester Old Spots	Large Black	Essex	Berkshire	Middle White	Large White × Large Black	Large White × Berkshire	Large White × Middle White
680—	—	—	—	—	—	—	38.0	—	—	—	—
700—	—	—	—	—	—	46.0	42.0	40.8	—	—	—
720—	43.5	—	—	—	—	—	52.0	48.1	—	—	—
740—	50.8	—	—	—	54.0	—	52.0	53.1	—	—	—
760—	52.5	53.8	—	—	57.0	59.3	60.8	61.3	—	—	—
780—	54.0	55.2	58.1	58.2	58.7	61.6	65.6	—	57.3	52.6	49.9
800—	56.0	60.8	61.1	65.1	64.7	66.3	69.4	—	54.8	54.8	54.6
820—	57.7	65.0	68.9	77.9	62.5	80.2	—	—	56.3	64.3	59.0
840—	65.0	—	62.6	69.5	59.6	79.6	—	—	59.8	—	—
860—	64.2	—	—	73.7	—	83.9	—	—	—	—	—
880—	81.5	—	83.5	—	—	—	—	—	—	—	—



Table XVII. *Belly thickness changes with increasing length of side in different breeds and crosses*

Bacon side length (mm.)	Belly thickness (mm.)										
	Large White	Long White Eared	Wessex	Gloucester Old Spots	Large Black	Essex	Berk- shire	Middle White	Large White x Large Black	Large White x Berk- shire	Large White x Midd White
680-	—	—	—	—	—	—	27	—	—	—	—
700-	—	—	—	—	—	33	29	31	—	—	—
720-	29	—	—	—	—	—	36	33	—	—	—
740-	31	—	—	—	34	—	36	36	—	—	—
760-	32	36	—	—	36	37	39	37	—	34	34
780-	32	36	36	35	36	39	40	—	35	35	37
800-	33	37	37	39	38	36	42	—	35	39	—
820-	35	40	42	39	40	41	—	—	37	—	—
840-	35	—	37	41	38	41	—	—	—	—	—
860-	35	—	—	42	—	44	—	—	—	—	—
880-	39	—	43	—	—	—	—	—	—	—	—
to Belly : back fat	1:1-15	1:1-32	1:1-23	1:1-30	1:1-38	1:1-18	1:1-08	1:1-18	1:1-08	1:1-08	1:1-11



## 428 *Body Proportions of Different Breeds of Bacon Pigs*

If pure breeds and the crosses between them are compared at constant length of side (see Table XII *b*), it will be seen that the weight of the bacon side in the cross-breeds is slightly less than the mean of the pure breeds. The back fat and belly thickness too is intermediate, except in the case of the Large White-Large Black cross, where the back fat is on the thin side of intermediate. This confirms the results obtained from grouping the sides according to weight of side (see p. 420 above).

### EFFECT OF SEX ON BODY PROPORTIONS

The effects of sex have been determined by a comparison of the proportions at constant body length (see Table XVIII).

Table XVIII. *Sex differences in weights and measurements at constant side length*

Breed	Sex	No.	Live lb.	Car-cass lb.	Bacon side lb.	Belly thick-ness mm.	Back-fat thickness (mm.)			Flank thick-ness mm.	Ratio shoulder fat loin fat
							Shoulder	Loin	Rump		
Large White	♂	52	190	146	55.5	31.5	50.4	25.9	37.3	20.8	1.95
	♀	52	188	147	54.4	34.7	48.3	24.5	35.7	21.0	1.97
Four breeds*	♂	34	200	158	58.6	32.9	56.6	33.7	46.3	22.9	1.68
	♀	34	190	148	54.6	35.3	53.7	32.8	44.1	21.4	1.64

\* Large Black, Long White Lop Eared, Berkshire, and Middle White.

At equal body lengths the sow has slightly lighter live, carcass, and bacon weights, but the belly is thicker and the back fat thinner than in the castrated boar pig. Murray<sup>(14)</sup> obtained much the same results as these from an examination of body measurements of the sexes of Large Black pigs in South Africa, as did Hansson & Bengtsson<sup>(11)</sup> in Swedish pigs. The reasons why the sow gives a carcass of better quality are probably twofold. In the first place, castration tends to the deposition of fat in the body, and in the second place the presence of ovaries in the sow tends to thicken the mammary gland region. That it is not due to a question of more early maturity of one sex than the other, is shown by the fact that the ratio of shoulder to loin fat (see p. 419) is approximately similar in each case. A few experiments were made at Onderstepoort to test these conclusions. Litters of Large Whites were divided into four groups—normal males and castrated males, normal females and castrated females—and killed at bacon weights.

As will be seen from the results in Table XIX the castrated boars and sows have thicker back fats than the corresponding normal animals, while the belly thickness is less in the castrated than in the normal sows.

Table XIX. *Effect of sex and castration on body measurements—Large White*

	No.	Live lb.	Car- cass lb.	Bacon side lb.	Belly thick- ness mm.	Back-fat thickness (mm.)			Flank thick- ness mm.	Ratio shoulder fat loin fat
						Shoulder	Loin	Rump		
Males	10	257	202	—	30.9	52.2	28.5	30.6	—	1.83
Castrated males	9	239	193	—	33.4	63.5	41.7	44.8	—	1.52
Females	11	221	173	—	35.6	58.5	34.3	38.2	—	1.71
Castrated females	9	243	195	—	34.2	66.2	39.5	45.9	—	1.68

Unfortunately not much can be done commercially to alter things if the above supposition is correct. In competitions and in the litter testing of bacon pigs for breed improvement, these facts should be taken into account.

#### SUMMARY

Weights and measurements of sides of bacon made under standard conditions from different breeds and crosses of pigs and exhibited at the London Dairy Show from 1922–31 have been analysed statistically.

1. The actual live weight of the pig affects the carcass percentage more than does breed or type.

2. For a bacon pig of 200 lb. live weight, there is an optimum carcass percentage (about 79 per cent) and an optimum bacon percentage (about 58 per cent). The highest percentage is not necessarily best, for below about this level the proportion of bone will be too large and above this level the proportion of fat will be too large.

3. The reasons why certain measurements and proportions are necessary to meet market requirements are detailed.

4. The changes in the proportions of the side with increase in weight of the side are described. The proportional increase in bacon weight is much greater than the proportional increase in side length. Both belly thickness and thickness of shoulder fat increase in their proportions at a faster rate than side length, but not so fast as side weight. The thickness of fat over the loin is the only part measured which increases at a faster rate than side weight.

5. The subcutaneous layer of fat matures earliest at the shoulder, over the rump next, and on the loin last. The rate of increase of the back fat slows down as the sides increase in weight. This slackening of growth is greatest in the shoulder and least in the loin, the rump area being intermediate.

## 430 *Body Proportions of Different Breeds of Bacon Pigs*

6. The ratio between the thickness of fat at the shoulder and at the loin decreases as the weight of the side increases. In early-maturing pork breeds the ratio decreases quicker than in the late-maturing breeds.

7. Since the region of the last rib is the latest maturing part of the body, it is at this place that the carcass should be cut in order to obtain a proper estimate of its development.

8. A comparison is made of the relative side weight, side length, back-fat and belly thicknesses of different breeds at constant side weight and at constant side length.

9. When first crosses between two pure breeds are compared at similar weights there is an indication that; while back fat and belly thickness are intermediate, the body length is slightly above the mean of the parent breeds.

10. For each unit of increase in side length the pork type increases much more in side weight than the bacon type does.

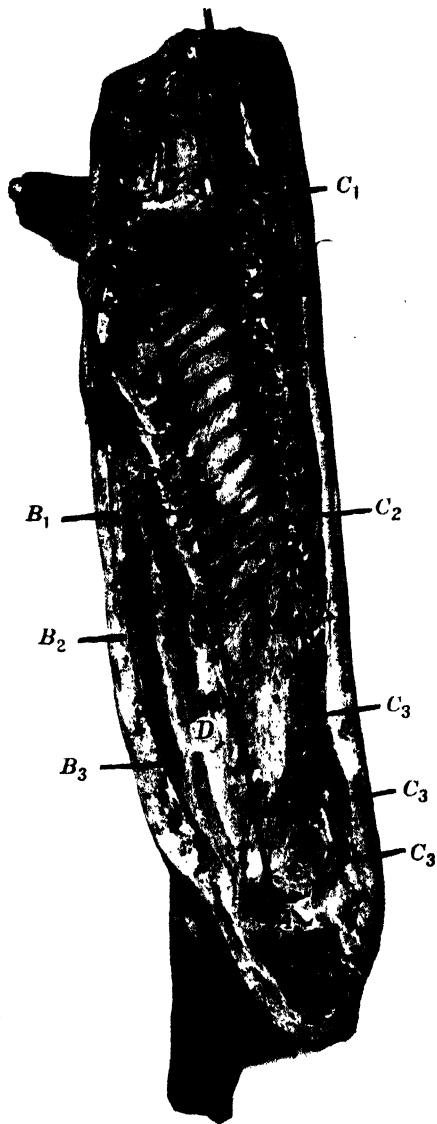
11. As the side is lengthened at constant side weight, so the thickness of the back fat is decreased, while there is no constant effect on belly thickness.

12. At equal side lengths the sow has a thicker belly and thinner back fat than the castrated boar pig.

13. Castration in either sex increases the thickness of the back fat, while in sows it also decreases belly thickness.

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Side of bacon showing where the measurements were taken.



Showing how the thickness of fat at the loin increases relative to the thickness at the shoulder as the pig matures (**1** and **2** Middle Whites from the same herd) and in early maturing as compared with late maturing breeds at the same carcass weight (**2** Middle White and **3** Large White).

(All carcasses reduced to similar shoulder fat thickness.)



1	2	3
Breed: Middle White	Middle White	Large White
Carcass weight (lb.) 52	111	120
Age (days) 101	141	144
Actual shoulder fat (mm.) 33	45	39
Actual loin fat (mm.) 11	31	23
Ratio $\frac{\text{Shoulder}}{\text{Loin}}$ 3.0	1.4	1.7



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# STUDIES ON THE RELATION BETWEEN CULTIVATION IMPLEMENTS, SOIL STRUCTURE AND THE CROP

## III. ROLLS: AN ACCOUNT OF METHODS EMPLOYED IN A STUDY OF THEIR ACTIONS ON THE SOIL

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(With Two Text-figures)

### CONTENTS

	PAGE
I. Introduction . . . . .	432
II. Laboratory studies	
(a) Tests of the distribution of compression in rolled soils by visual methods . . . . .	433
(b) The measurement of pressure in soils and other granular materials . . . . .	436
III. Field studies	
(a) Tests of consolidation, moisture content and tilth . . . . .	441
(b) The measurement of resistance to the penetration of the soil by a steel probe . . . . .	445
IV. Summary . . . . .	446
References . . . . .	446

### I. INTRODUCTION

THE origin of the practice of rolling is, like that of many other cultivations, somewhat obscure, but the first use of the roll must be assigned to a later period of agricultural history than that of such implements as the plough and harrow. It is probable that the crushing of clods was the main object when rolls were first employed. Before they came into general use, clods were broken by being struck with a small mallet called a "maul". The first rolls were probably made entirely of wood; stone rolls followed, and these have in turn been generally superseded by iron ones. There exists to-day a great variety of types and sizes, but the two types most commonly used in England are the "flat" roll and the ribbed "Cambridge" roll. Rolls are now put to a variety of uses, and in some cases the benefits attending their use are not clear. Differences of opinion exist among farmers concerning the effects produced by such common operations as the rolling of grassland and of autumn-sown

corn in spring. The spring cultivation of winter wheat is dealt with in a paper by Garner & Sanders (1).

It is clearly important to determine when and how rolling is beneficial. Although it is one of the simplest of all cultivations, there can be no doubt that time, effort and money are sometimes wasted on rollings which are useless or even harmful. Little progress can be made in the study of any cultivation without careful determinations of the state of the soil before the operation is performed, and of the precise effect of the cultivation on the soil. With such determinations, it may then be possible to relate the cultivation and the development of the crop. It is, therefore, necessary to be able to measure and record the effects of rolling on soils.

This paper deals mainly with methods which have been used to determine the effects of rolls on soils in the laboratory, and with the application of some of these methods to field experiments.

## II. LABORATORY STUDIES

### (a) *Tests of the distribution of compression in rolled soils by visual methods*

(i) *The use of plaster casts.* A light sandy soil, sifted through a sieve of 3 mm. mesh, was built up in a specially constructed box in uniform layers, each 1.3 in. deep, separated from one another by thin sheets of tinfoil. Five such layers, of the same uniform thickness and compacted to the same degree, were superimposed. A metal drum, 16 in. in diameter and weighing 160 lb. per foot of length, was then placed on the top layer of soil. After removal of the drum, the top layer of soil was cleared away and plaster of Paris was poured into the depression lined by the first sheet of tinfoil. When a cast of this depression had been formed and removed, the other layers of soil were in turn cleared away, and casts made of the depressions in a similar way.

When the casts were dry they were cut in halves, and the vertical heights of the depressions at the centres were measured. From these heights the compression of the various layers could easily be calculated. Table I contains some results obtained with the same soil at three moisture contents. The main difficulty in this, and in all laboratory work in which the behaviour of soils at different moisture contents is investigated, is to vary the moisture without altering other conditions, notably the initial compactness of the soil. There is no completely satisfactory method of doing this. One of the best methods is to start with soil of

## 434 *Cultivation Implements, Soil Structure and the Crop*

the same moisture content in each case, to pack it in thin layers by a standard procedure, and either to spray each layer with the required quantity of water in the form of a fine mist, or to dry out the layers of soil very gradually. With the most careful technique, however, it is impossible to obtain a uniform distribution of moisture throughout the mass of soil. Veihmeyer<sup>(2)</sup> and others have experienced similar difficulties.

Table I. *Table showing compression of a sandy soil\* at different depths and moisture contents. (Figures represent the compression (in.) of layers of soil 1.3 in. thick)*

Soil layer	Soil moisture (% of the moist soil)		
	14.7	7.0	2.9
1st layer (0-1.3 in.)	0.6 in.	0.3 in.	0.1 in.
2nd layer (1.3-2.6 in.)	0.45 in.	0.2 in.	0.05 in.
3rd layer (2.6-3.9 in.)	0.25 in.	0.1 in.	—
4th layer (3.9-5.2 in.)	0.1 in.	—	—

\* Mechanical analysis: coarse sand, 64 per cent; fine sand, 10 per cent; clay, 9 per cent. This soil was used for all the laboratory studies here described.

The results show that the compressing action of the given cylinder was greater on moist than on dry soil. This is, of course, in accord with common observation. A study of the distribution of compression at different depths shows that compression is inversely proportional to depth, to a first approximation. This result is in accord with the findings of Nichols<sup>(3)</sup> who has studied the compression produced when a cylindrical plunger is forced into the soil. When layers of moist and dry soil were used together, e.g. moist soil at the top and dry at the bottom, or dry at the top and moist at the bottom, the compression in each layer was influenced by its moisture content and position, and partly also by the other layers with which it was in contact. For example, when the upper layers were composed of dry soil and the lower of moist, the compression in the lower moist layers was usually slightly greater than when all the soil was moist; when moist soil was at the surface and dry soil below, the compression was concentrated mainly in the upper layers.

(ii) *The fixation of prepared soils.* A sieved soil was built up in uniformly compacted layers, each 1 in. thick, in a large box measuring 6 × 2 × 1 ft. deep. The layers were separated from one another by thin layers of chalk. A short length of a Cambridge roll, comprising five 3 in. wide sections of 16 in. diameter, was then drawn over the soil. Blocks of the soil were removed and fixed in a mixture of paraffin wax and naphthalene by a method which has been described by Mistschenko<sup>(4)</sup>. Blocks were removed from the rolled and the unrolled soil, and those

taken from the unrolled soil showed that the method of building up the layers was reasonably efficient after a few trials had been made. Fig. 1 illustrates results obtained with a block taken from the sandy soil previously mentioned, at a moisture content of 13.3 per cent of the moist soil. Results obtained at various depths and moisture contents were in general accord with those obtained by the method described in the previous section. A notable feature was the shallow depth at which no

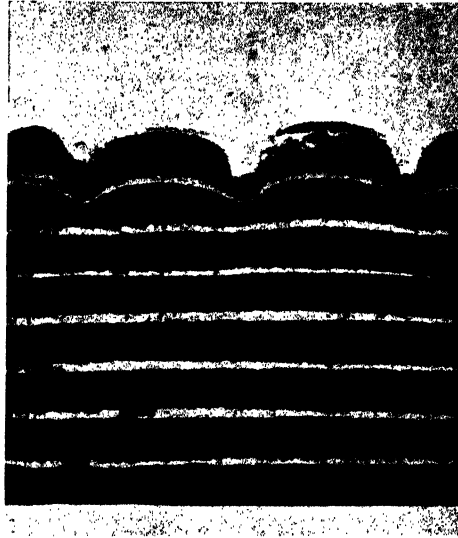


Fig. 1. Section of a block of (a prepared) sandy soil, rolled with a Cambridge roll and fixed by impregnation with a mixture of paraffin wax and naphthalene.

visible compression was produced. In many cases, especially with drier soils, there was no visible effect at depths greater than 2-3 in. from the surface. It should not be inferred, however, from this statement, that the Cambridge roll produces no effects at depths greater than this. Tests by other methods have shown that the roll may exert effects at depths where no compression is visible by this method. For example, in the case of the test illustrated by Fig. 1, Table II shows the effects of the rolling on the resistance to the penetration of a steel probe (*vide* § III (b)) at various depths.

Table II. *Table showing mean resistances (lb.) to penetration of a steel probe at various depths on rolled and unrolled soil. (Soil at 13.3 per cent moisture, and prepared as in Fig. 1)*

Depths (in.) ...	1	2	3	4	5	6
Not rolled	3.4	3.5	3.7	3.8	3.8	4.1
Rolled	8.3	9.2	7.5	5.0	4.5	4.3

(b) *The measurement of pressure in soils and other granular materials*

(i) *With pressure gauges.* It is readily apparent that any form of pressure gauge which measures pressure by the displacement of fluid from a capsule into a manometer, must fail to give a true measure of pressure within a granular material because of the arching of the material over the capsule.<sup>1</sup> For example, consider a cylindrical capsule, fitted with a diaphragm and filled with a fluid which is connected by tubing to a pressure recording device. An increase of pressure on the outside of the diaphragm causes it to move inwards, and the greater the increase in pressure, the further the diaphragm must move. If such a capsule is used for measuring pressure in soil, the soil is hindered by arch action<sup>1</sup> from freely following the movement of the diaphragm. The smaller the distance the diaphragm moves under a given pressure, the more accurate is the measure of that pressure. The ideal is a gauge in which the diaphragm does not move at all, an increase of pressure on the outside causing an equal increase inside the capsule through the operation of an automatic electrical device. Such gauges have been employed by Nichols(3) in fundamental studies on pressure in soils, but they are not adapted to the measurement of the rapidly fluctuating pressure caused by the passage of a roll. It was therefore decided to use a fairly simple gauge to obtain an indication of the pressures in rolled soils, since it has been shown by Jenkin(5) that true pressures in granular materials are indeterminate, however refined the apparatus.

The most satisfactory type of pressure gauge used consisted of a disk-shaped steel capsule, fitted with a thin steel diaphragm and connected by flexible metal tubing to a mercury manometer. Steel was chosen for the diaphragm after trials with rubber, leather, oiled silk and brass. The diaphragm was soldered on to the capsule, which was 2 in. in diameter and  $\frac{1}{4}$  in. deep. Alcohol was used in the capsule and mercury in the manometer, and the apparatus was so constructed that all air

<sup>1</sup> An account of some of the phenomena associated with arch action is contained in the Rede Lecture by Osborne Reynolds: *On an Inversion of Ideas as to the Structure of the Universe*. Cambridge University Press, 1902.

could be excluded from the system. The reason for the use of an alcohol-mercury system was mainly that the displacement of the whole capsule, which sometimes occurred during the rolling operation, had a much reduced effect on the manometer zero when the lighter liquid was used. The apparatus was very sensitive. With capillary tubing of 0.8 mm. bore in the manometer, movement of the diaphragm was only slight even when large pressures were recorded.

The pressure gauge is of limited usefulness, but certain general conclusions may be drawn from its use. Table III shows some results obtained when the capsule was used to measure the effect of the Cambridge roll on the sandy soil in the box previously mentioned. The figures in Table III should not be given undue significance, for they are

Table III. *Table showing the increased pressure produced in a capsule buried in a light sandy soil, when the soil was acted upon by a Cambridge roll. (At various depths and moisture contents.) Pressures in cm. of mercury*

Moisture (% of moist soil)	Depth of capsule (in.)	(1) Covered with soil	(2) Roll passes over first time	(3) Steady pressure after first roll	(4) Roll passes over second time	(5) Steady pressure after second roll	(6) Steady pressure after tenth roll
15.6	2	0.6	37.0	1.2	32.0	1.4	2.4
	4	0.9	15.1	2.1	19.3	2.7	4.1
	6	1.0	13.6	2.6	14.6	3.0	3.8
	8	1.2	10.8	2.4	15.6	3.2	4.0
12.7	2	0.4	26.2	0.8	22.2	1.2	2.4
	4	0.9	22.5	2.1	14.5	2.3	4.7
	6	1.4	19.2	5.2	18.6	5.8	8.8
	8	1.6	13.2	6.6	14.4	7.4	9.2
12.3	2	0.3	25.3	0.9	24.1	1.5	3.3
	4	0.6	12.6	2.0	14.8	2.6	4.4
	6	0.8	11.2	3.2	10.4	3.6	5.0
	8	1.4	9.2	4.8	10.2	5.6	7.4
10.3	2	0.2	19.8	0.4	21.8	0.6	0.8
	4	0.4	14.2	1.4	12.6	2.0	3.8
	6	1.0	12.4	3.0	12.4	3.4	5.0
	8	1.0	11.0	3.6	12.2	3.8	5.6

the results of experiments which were incapable of exact repetition. The following general conclusions, however, may be drawn from these and other experiments: (1) the effects of rolling on the soil can be detected at great depths (greater than Table III shows) with this apparatus; (2) during the passage of the roll over the gauge, the pressure rises to a maximum and then falls off after the roll has passed; (3) when the gauge is near the surface, the maximum pressure is very high, but the final

## 438 *Cultivation Implements, Soil Structure and the Crop*

pressure after the roll has passed is low; at greater depths, the maximum pressure recorded is not so high, but the final pressure after the roll has passed may be higher than in the upper layers. The differences in maximum pressures are readily explained, for it is shown in § II (b) that the pressure produced by a weight situated on the surface of a granular material falls off rapidly with increasing depth. When the weight of the roll is removed, the pressure set up within the capsule exerts a force on the diaphragm which, when no longer opposed by an equal force on the outside, causes the soil above the capsule to be displaced upwards, so allowing the pressure within the capsule to fall. When the capsule is buried deeply in the soil, the tendency of the capsule diaphragm to move upwards after the roll has passed over is opposed by the weight of a greater volume of soil, which may be cemented into a solid mass; (4) if the roll is pulled over the buried gauge at high speed, neither the maximum nor final pressure is as great as at low speeds.

(ii) *Measurement of the friction between soil and metal.* Owing to the difficulties bound up with the phenomenon of "arching", the use of the capsules described above does not give a true measure of the pressure in a granular material. One of the most reliable estimates of pressure may be obtained by measurement of the static friction between the soil and metal wires, rods or cylinders buried in it. Jenkin<sup>(5)</sup>, who has dealt at length with the mathematics of pressure in granular media, has shown that true pressures in such media are indeterminate. But it is considered that the methods described below give indications of the pressure which are nearer to the true pressure than the results obtained by pressure gauges. The objects of the experiments described were to obtain comparative indications of the pressure produced in different conditions, and to attempt to find how the pressure was distributed through soils.

A series of experiments was carried out in the laboratory in a large box of soil. The box was fitted at the ends and sides with slots through which wires or rods could be passed in a horizontal direction. The rods or wires could be placed at various depths below the surface of the soil, which could then be rolled by the short length of Cambridge roll already mentioned. A measure of the pressure at various depths before and after rolling was then obtained by pulling the rods or wires very gradually, through a spring balance, by means of a small winch. The force required to initiate movement of the rods or wires was recorded, and values thus obtained may be taken as indications of the pressure exerted on the metal by the soil. The figures are a measure of the adhesion between the soil and metal. They give an indication of the effects which might be pro-

duced by rolling on the roots of plants, and show to what extent it may be possible to increase the firmness of roothold. Table IV shows the results obtained with the sandy soil at various depths and moisture contents.

Table IV. *Table showing the force required to initiate movement in 6 ft. lengths of  $\frac{1}{4}$  in. diameter brass rod when buried at various depths in soil of varying moisture content, and rolled with a Cambridge roll. Pulls in lb.*

Moisture %	Depths of rods (in.)	Before rolling	After 1st roll	After 2nd roll	After 10th roll
15.6	1	2.1	10.5	16.5	33.0
	3	2.1	15.6	19.5	27.0
	5	3.6	15.0	18.0	27.0
	7	3.1	13.4	15.0	21.0
12.7	1	0.9	10.5	12.0	36.0
	3	1.2	20.1	23.1	34.5
	5	1.5	16.5	21.0	27.0
	7	4.4	13.4	16.5	19.5
12.3	1	1.5	7.5	7.5	22.5
	3	3.0	18.6	24.0	34.5
	5	3.6	19.5	20.1	27.0
	7	4.9	17.0	18.0	24.0
10.3	1	2.1	6.0	6.0	18.0
	3	3.0	14.1	16.5	25.5
	5	3.0	16.5	16.5	20.0
	7	3.6	15.0	16.0	19.5

The figures in Table IV may be contrasted with those obtained in similar conditions in Table III, but with the proviso that the figures should not be regarded as exact ones which could be repeated at will. The results may be briefly stated in general terms as follows: (1) the effects of rolling were easily demonstrable by this method to depths of 1 ft. in loose soil; (2) the effects produced by rolling increased with increasing moisture content over the range at which the experiments were performed; (3) when layers of soil of different moisture content were experimented upon together (in other experiments) the pulls recorded were mainly influenced by the condition of the soil immediately surrounding the rods; (4) it should be noticed that the highest pulls recorded after rolling usually occurred at the 3rd inch. This may be contrasted with the results shown in Table IV, where the highest residual pressures were usually at the 6th or 8th inch. It is reasonably certain that measurements of pressure by the friction method are more reliable than those obtained with the pressure gauge, owing to the movements of the gauge diaphragm already mentioned.

The measurement of pressure by means of the rods was not, however,



## 440 *Cultivation Implements, Soil Structure and the Crop*

entirely satisfactory, even for laboratory studies, for under similar conditions it was difficult to obtain results which were in good agreement. The difficulties were due partly to the inevitable bending and displacement of the rods or wires which occurred when the rolling was carried out. This made it difficult and sometimes impossible to obtain sharp readings for the limiting friction values. It was decided therefore to employ the method described below for further experiments.

(iii) *Measurement of friction on the surface of a rotating cylinder.* A brass cylinder, 2 in. in diameter and 2 in. long, was attached to one end of a  $\frac{1}{4}$  in. diameter rod, 15 in. long, which had a pulley attached to the other end. The rod was supported on ball bearings inside a  $\frac{7}{8}$  in. diameter tube. A small winch was attached to the tube in such a way that the cylinder could be gradually turned by applying a pull to a cord which passed round the pulley. A spring balance between the winch and the pulley gave a measure of the force required to turn the drum.

The drum was buried in various positions in the experimental box by passing the tube through holes drilled in the sides of the box. When the drum was buried in the required position and an estimate of the pressure in that position was to be obtained, a force was gradually applied to the drum by slowly turning the winch. It was found that very sharp readings of the static friction could be obtained with a proper procedure. The general experience was that the pull registered on the spring balance gradually increased until the limiting value was reached; the drum then suddenly rotated a little, and the pull fell until the winch was turned again. This apparatus was used for measuring the distribution of "pressure" in a box of pure dry sand. The behaviour of pure sand is more simple than that of soils, and a test of the utility of the friction apparatus was made with this material. A brief account of the studies serves to illustrate the nature of some of the problems encountered when attempts are made to measure the effects of implements on the soil.

When dry sand is poured, haphazard, into a box, there is a very varied packing of the grains. In some parts the grains are closely packed, and in others there are "pockets" where packing is very loose. Tests with the automatic mechanical resistance recorder (*vide* § III (b)) clearly demonstrate this irregularity. (Similar irregularities are found in the most uniform stretches of sand on the seashore.) If the box of dry sand is shaken, the grains settle down to a closer state of packing. On continued shaking, the contraction in volume is at first rapid, then more gradual, and finally becomes inappreciable. No method of shaking, pressing or tapping which resulted in a uniform state of packing could

be found after a trial of such methods as (1) raising the box by means of a large counterbalanced lever and allowing it to drop with a more or less severe shock; (2) shaking the box by means of a rotating cam; (3) tapping the bottom of the box by means of a powerful electromagnet and rotary converter. A variable degree of success was achieved by the use of these and other methods, but none gave a box full of sand in such a uniform condition that any accurate experiments on the distribution of pressure could be attempted.

A method finally adopted was to experiment with the sand in a very loose condition by allowing it to flow through a fine screen and avoiding all shaking. It was possible, with an elaborate procedure, to obtain readings with the friction cylinder of the pressure at various places in the box of sand (*a*) when no load was applied to the surface, and (*b*) when a length of roll was placed on the surface. The distribution of the pressure in a 2 ft. cube of sand was roughly traced, but it was found that the distribution was appreciably influenced by the sides and bottom of the box. Experiments with moist sand introduced further complications which could not be dealt with in a completely satisfactory manner, and it does not seem likely that *accurate* experiments on the distribution of pressures in moist soils can ever be made with the use of buried gauges or friction cylinders, even with the most careful technique. The use of the friction cylinder is not easily adaptable to field studies, since it is always necessary to prepare the soil and bury the cylinder beside an observation trench.

### III. FIELD STUDIES

#### (*a*) *Tests of consolidation, moisture content and tilth*

A mild-steel tube, 12 in. long and of internal diameter 3 in., was sharpened at one end. Cuts were made half-way through it at distances of 1, 2, 3, 4 and 5 in. from the sharpened end, and a removable handle was attached to the other end (Fig. 2 *a*). Steel plates (Fig. 2 *b*), when pushed into position in the cuts made in the tube, divide the lower part of the tube into small compartments, each 1 in. high and 3 in. in diameter. This tube may be used for taking samples of the various layers of the soil in field experiments. To obtain a sample, the tube is forced into the soil until the top cut is level with the soil surface. It is then withdrawn, with a core of soil inside it. The plates are driven into the core, dividing the soil into small cylinders of equal dimensions. These cores may be removed in turn from the tube and placed in air-tight tins for deter-

minations of the apparent specific gravity, moisture content or tilth of the various layers.<sup>1</sup>

This tube is a useful device for sampling most soils which are reasonably free from stones, but in soils which are either very loose or sticky, the core may be compressed so much that the determinations are worth-

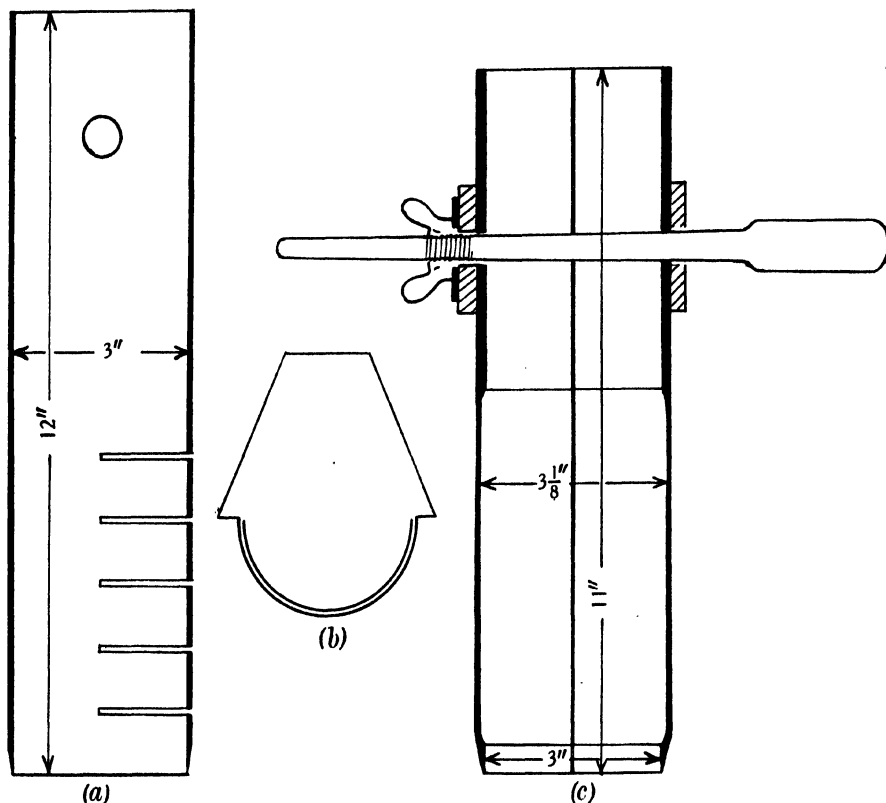


Fig. 2. (a) Tube for sampling soil inch by inch; (b) plates for use with tube a; (c) split tube for use on wet heavy soils.

less. A split tube (Fig. 2 c) with a cutting edge of a slightly smaller diameter than the rest of the tube has been successfully used on wet heavy soils. In this case, the tube is opened longitudinally to remove the core, which may then be cut as desired.

The sampling tube described has been employed in many cultivation experiments for the purpose of determining the effects produced on the

<sup>1</sup> My thanks are due to Mr E. J. H. Berwick, B.A., for invaluable help in the development of this method of sampling.

soil by the cultivation implements. Its use for this purpose may be illustrated by the results obtained in an experiment on the spring cultivation of autumn-sown wheat, carried out on a black Fen skirt soil in 1935-6. The land on which the experiment was laid down was a good skirt soil in high condition, with a permanent water table about 4 ft. from the surface. The crop was Yeoman wheat after potatoes. In February the surface of the soil was very loose, and the wheat plants had such a poor roothold that they could easily be pulled out of the ground, complete with their larger roots. The treatments carried out were: (1) roll in February, (2) roll in February, horse-hoe and harrow twice in April, and (3) control, no spring cultivations. Tests were made in May with the soil sampler described, and some of the results are presented in Table V.

Table V. *Table showing the soil conditions on 26. v. 36 in a spring cultivations experiment carried out on a Fen skirt soil in 1935-6*

Observation	Depths (in.)	Control. No spring cultivation	Rolled in February	Rolled in February; Horse- hoed and harrowed in April	Significant differences	
					$P < 0.05$	$P < 0.01$
(a) Weight of dry soil (g.) per cu. in.	1	13.20	15.73	15.42	1.82	2.55
	2	11.93	16.10	15.39	1.27	1.78
	3	12.70	15.43	15.44	1.58	2.22
	4	12.77	14.38	14.36	Not significant	
(b) Weight of water (g.) per cu. in.	1	4.13	5.61	5.50	0.77	1.08
	2	4.14	6.20	6.17	0.79	1.10
	3	4.93	6.22	6.20	0.93	1.31
	4	5.13	5.97	5.87	Not significant	
(c) Moisture as per cent of the moist soil	1	23.9	26.3	26.3	Not significant	
	2	25.8	27.8	28.6	1.75	—
	3	27.8	28.6	28.6	Not significant	
	4	28.5	29.2	28.9	Not significant	
(d) "Surface" figures (relative)	1	100.0	79.2	83.7	4.8	6.9
	2	81.8	39.5	37.3	16.6	23.2
	3	59.8	33.4	28.1	19.5	27.3
	4	40.5	28.4	29.0	Not significant	

*Consolidation.* The relative figures obtained for apparent specific gravity by means of the sampling tube may, within the limits of a properly laid out experiment, be taken as an indication of the consolidation on the variously treated plots. Rolling of a loose soil produces a consolidation which may extend to depths of up to 6 in., but when soils which have settled over the winter are rolled in spring, there is rarely any appreciable consolidation at depths greater than 3 in. Table V (a) shows the order of the consolidation produced by rolling

#### 444 *Cultivation Implements, Soil Structure and the Crop*

winter wheat on a particular skirt soil with a Cambridge roll, 24 in. in diameter and weighing approximately  $2\frac{1}{2}$  cwt. per foot of width. Results obtained for consolidation by use of the tube in laboratory studies agree well with those obtained by the visual methods described in § II (a).

*Moisture.* Tests of the effect of rolling on the moisture content of field soils have rarely shown any significant differences between rolled and unrolled soils if the moisture is expressed as a percentage of the moist or dry soil. But rolling, by reducing the air spaces in the soil, has the effect of increasing the moisture contained in a unit volume of soil. This increase in the gross moisture content of unit volume of a rolled soil is well illustrated by Table V (b). The tests which yielded the figures presented in Table V were carried out about 3 months after the rolling operation. Other experiments suggest that the effect of rolling on the gross moisture content of unit volume of the soil usually persists as long as the increased consolidation.

A review of an extensive literature concerning the effects of cultivations on soil moisture shows many conflicting results. It was generally accepted until a few years ago that rolling helped to raise water to the surface by increasing the capability of the soil to draw water from below by capillary action. It has, however, been shown that the actual transference of water in soils by capillary action could never be capable of drawing up water from a water table existing at a great distance below the surface. Table V (c) shows that, at the time when the tests were carried out, there was a higher percentage of moisture at a depth of 2 in. on those plots which had been rolled than on those which had not. This may possibly be accepted as evidence of the rise of moisture from the water table 4 ft. below, but this is a completely isolated result, and it seems likely that it was due to the greater capacity of the rolled soil to retain in that particular layer some of the rain which had fallen a few days previously.

*Tilth.* A measure of the average sizes of the soil crumbs may be obtained by performing sieving tests on samples obtained by means of the sampling tube described. Though sieving tests are generally more useful if carried out immediately on the field, useful data may sometimes be obtained with samples which have been dried for the determination of the consolidation and moisture content. For the latter purpose, a set of japanned metal sieves, 8 in. in diameter, 2 in. high and fitted with a lid and plain bottom have been used. The meshes used are (1)  $\frac{3}{8}$  in., (2)  $\frac{1}{2}$  in., (3)  $\frac{5}{8}$  in., (4)  $\frac{1}{4}$  in. and (5) plain bottom.

When the effects on soil tilth of a number of cultivation treatments

are to be compared, it is convenient to express the tilth of each sample by means of a single figure. One of the most satisfactory measures of tilth is a figure which is proportional to the "surface area" of the crumbs. Keen<sup>(6)</sup> has explained the use of such a "single value" figure for the total fragmentation of soils. Whether the crumbs be considered as spheres or as cubes, the surface area of a given weight of soil is inversely proportional to the diameters in the case of spheres, and to the lengths of sides in the case of cubes. Soil crumbs are neither cubes nor spheres, but it is reasonable to suppose that fairly reliable relative figures for the surface areas of samples may be obtained by finding the sum of the products of the percentage of each fraction and the reciprocal of the mesh of the sieve above. For example, the relative figures presented in Table V (*d*) were obtained by finding the sum of the percentage of the first fraction, twice the percentage of the second, four times the percentage of the third, eight times the percentage of the fourth and sixteen times the percentage of the last fraction. It may be argued that 1 and 16 are not the correct "index" figures for the first and last fractions, but since the figures are only relative, this objection is of little importance. It has been suggested from time to time that similar single figures should be used to denote the texture of soils as indicated by their mechanical analyses. The objection to the use of a single figure for this purpose is that the same figure may apply to two or more very different types of soil. But this objection does not apply to the use of a single figure in the present instance, where the effects of different cultivations on the same soil are compared, the experimental treatments being randomized over the area.

Tests of the effects of rolling on soil tilth by this method, both in the laboratory and in the field, have yielded results which accord well with other measurements. In some cases, the roll breaks down clods on the surface of the soil and decreases the average size of crumb there. At other times, when the soil is moist, rolling may cause the soil crumbs to adhere and form large clods. This effect is particularly noticeable when winter-sown crops are rolled in spring, if the soil is moist at the time of rolling. Table V (*d*) shows the effects produced at various depths by a spring rolling on the Fen skirt soil previously mentioned.

*(b) The measurement of resistance to the penetration of the soil by a steel probe*

The apparatus described in a previous paper<sup>(7)</sup> has been found useful in studies of the effects produced by rolls, especially in field conditions. It gives a fairly reliable measure of the "firmness" of the soil, and pos-

## 446 *Cultivation Implements, Soil Structure and the Crop*

sesses the advantage that no special preparation is required for its use and that it does not involve serious interference with the growing crop. It has given results which are in general agreement with the figures obtained for consolidation by other methods, but there is, of course, no simple relationship between the figures obtained for mechanical resistance and for consolidation. No such relationship could exist, owing to the large number of variable factors, including moisture content, by which the resistance values are influenced.

### IV. SUMMARY

An account is given of methods which have been employed in laboratory and field studies of the actions of rolls on the soil. These studies have revealed little that is remarkable or unexpected concerning the actions of rolls on the soil, but they have clearly demonstrated that the exact actions of rolls differ widely according to the circumstances in which they are employed. When rolling is carried out in field experiments, it is necessary to perform tests to measure the exact effects produced on the soil. In this way it should gradually become more apparent when and how rolling is likely to be beneficial.

Of the methods which have been described, the last two, viz. tests of the consolidation, moisture content and tilth by means of a sampling tube, and of the mechanical resistance to the penetration of a probe with the automatic "resistance" recorder, are well adapted to use in field experiments. These methods are being used in many field experiments involving cultivations, and it is hoped that it may at some time in the future be possible by such methods to relate cultivations and crop yields through a knowledge of the soil structure.

I am indebted to Mr F. H. Garner, M.A., Mr H. H. Nicholson, M.A. and Dr H. G. Sanders, M.A., for help and co-operation in these investigations, and to Prof. F. L. Engledow, C.M.G., M.A., for advice at all stages of the work.

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# ON THE SPRING CULTIVATION OF AUTUMN-SOWN WHEAT

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## INTRODUCTION

TIME-HONOURED custom demands that autumn-sown wheat should be rolled and harrowed in spring; this may be regarded as part of the art of farming, to be carried out or not according to the conditions at the time, but in actual fact a very large proportion of the autumn-sown cereal area of the country is so treated. Nevertheless, the practice lacks experimental justification. As is usually the case with experimental work on cultivations, the efficacy of the treatment must depend to a very high degree on the conditions as regards soil, crop and weather; this makes any enquiry into the subject very complex and requires wide repetition of experiments, both in time and in place. The present paper cannot cover the whole range of conditions, including as it does only the results of experiments carried out on the Cambridge University Farm; work on other soil types is still proceeding.

The soils of the Cambridge University Farm fall fairly distinctly into two different types. Three of the eight experiments described in this paper were located on light soil derived from an old river gravel overlying gault clay, and containing very varying proportions of stones, sand and clay, with very little silt. This soil type can only be described as light, but drainage in places is not very free and some surface capping often occurs. The other five experiments were situated on really heavy gault clay, which analysis has shown to be among the heaviest soils of the country.

In general practice the usual sequence is the roll followed by the harrow, the chief exception being when the autumn seed bed was very rough, in which case it is considered best to distribute the weathered clods with the harrow before rolling. The chief object of rolling is to press the soil firmly around the roots of the plant, on the same principle that leads a gardener to "heel in" his plants in the spring of the year. It must be explained that, apart from that produced by horses' hoofs or



## 448 *On the Spring Cultivation of Autumn-sown Wheat*

tractor wheels, consolidation given by the roll is much less than that obtained by the human heel. Measurements have shown that, on the two soil types with which this paper is concerned, the consolidating effect extends to a depth of only 2 in. below the surface (1). Firmness is reputed to benefit the plant in a variety of ways; it is claimed that, by ensuring close contact between root hairs and the soil, strong growth and tillering are promoted and "lodging" reduced. Rolling may have a useful effect in controlling weeds (1), a fact which at first sight is surprising; at that time many weed seeds lying close to the surface are germinating and the displacement caused by rolling may kill many of them. It is by no means uncommon for some capping to occur during the winter, and a ring roll is particularly efficient in breaking down this cap, which crumbles under it. In extreme cases the surface cap might inhibit the passage of oxygen and carbon dioxide to and from the soil, or even lead to mechanical damage of the plant; breaking that cap will at least allow rain water to enter the soil. The old view was that rolling had an important effect in facilitating the rise of soil moisture by capillarity, but this view is not now universally held. Many farmers regard rolling as the only means of countering wireworm attack in spring; the experiments here described gave no information on this point, because no wireworm attack was encountered. Finally, the roll has the obvious effect of levelling the surface; this may be important at harvesting in reducing draught and wear and tear of machinery.

The harrow also may play an important part as a leveller. The main claims advanced in its favour, however, are that it controls weeds and produces a surface mulch. Its effect on weeds is twofold. The passage of the tines through the surface soil causes sufficient disturbance to kill many seedlings, especially when they are just germinating. The weather may not permit of harrowing at the precise time when the majority of the seedlings are in the vulnerable stage, but when the operation is carried out at that time spectacular results may be obtained, especially in the case of poppies. Harrowing may also have some effect on established weeds like *Stellaria media* and *Veronica* spp., a certain proportion of which may be pulled out of the soil; in this connexion it should be pointed out that the harrow will tear off some tillers and remove some whole wheat plants, but the advantage or disadvantage of this must clearly depend on the thickness of the crop. As pointed out above, opinion is not agreed upon the advantages of a surface mulch.

Spring cultivations may therefore vary considerably in their value according to the conditions under which they are carried out, and their

effect on final yield must be anything but constant; this calls for long and continued experimentation. It may be argued that the cost of these operations is trifling, but in view of their wide use in the country it is worth while attempting to determine what benefit they confer on the crop, or at least to establish the fact that they do no harm.

#### DESCRIPTION OF THE EXPERIMENTS

During the years 1930-6 eight experiments were carried out on the University Farm at Cambridge, to measure the effect of rolling and harrowing autumn-sown wheat in spring. In general, rolling and harrowing have been tested separately and together, but in the first year, when there were two experiments, the operations were not tested separately. In five of the experiments the enquiry was combined with other studies, the layout being confounded, but in no case were the effects of spring cultivations influenced by other treatments; these latter are not, therefore, dealt with in this paper.

Some of the experimental details of the experiments are given in Table I.

In exp. Light I, the cultivations were superimposed on a Latin square; in exps. Light II, Light III and Heavy IV they were tested on the main plots of a randomized block layout; in exp. Heavy VI they were tested within the main plots of a randomized block experiment; in exps. Heavy V, Heavy VII and Heavy VIII, only the spring cultivations were studied, and the layouts were simple randomized blocks. This variation in layout has been partly responsible for the variation in size of plot shown in Table I.

Exps. Light I, II and Heavy IV were designed for binder harvesting, but experience of the method was unfavourable and the later experiments were harvested with a sickle. Cutting with a binder makes it very difficult to take random samples at harvest for detailed study (size and number of ears); in the binder-harvested experiments samples were restricted to the ends of the plots, and were, therefore, practically useless in explaining yield differences which were determined with greater accuracy from the produce of the whole plots. In the case of the exp. Heavy VI, the same limitations applied, the samples being restricted to a separate area along one side of the experiment. In the case of the remaining four experiments the samples were randomized all over the plots and were therefore truly representative.

There is an old saying among heavy land farmers that "a March roll makes an April fool", but the time at which these operations are carried

Table I. *Description of experiments*

Year	Soil type and no. of exp.	Size of plot acres	No. of repli- cations	Previous crop	Variety of wheat	Date of spring cultivations	Mean yield per acre		Remarks
							Grain bush.	Straw cwt.	
1930-1	Light I	1/16	8	Potatoes	Little Joss	24 Mar.	45-60	71-67	Partly lodged. Spring culi- vations had no effect on lodging, but significantly re- duced "foot-rot"
1931-2	Light II	1/17	7	Potatoes	Little Joss	17 Mar.	35-09	53-74	Half experimental area lodged. Harrowing significantly in- creased lodging. Roll pro- duced no effect on lodging
1932-3	Light III	1/16	6	Potatoes	Little Joss	27 Mar.	35-04	30-02	No lodging
1930-1	Heavy IV	1/16	12	Beans	Wilhelmina	26 Mar.	32-97	37-44	No lodging
1932-3	Heavy V	1/97	10	Sainfoin and bastard fallow	Wilhelmina	27 Mar.	66-43	73-40	Whole experimental area com- pletely lodged just before harvest
1933-4	Heavy VI	1/97	12	Oat and tare, hay and bas- tard fallow	Wilhelmina	5 Apr.	57-51	62-20	Some lodging but not asso- ciated with treatments
1934-5	Heavy VII	1/97	10	Silage and bastard fallow	Wilhelmina	29 Mar.	49-18	66-33	Crop half lodged—lodging not affected significantly by treatments
1935-6	Heavy VIII	1/161	10	Silage and bastard fallow	Wilhelmina	25 Mar.	48-82	62-16	Whole crop lodged flat

out must depend on the condition of the soil and crop, and on the weather, rather than on the calendar. Although in seven of the eight experiments the cultivations were performed in March, in every case the time selected agreed with local practice. Care was taken to ensure that the land, both on the surface and to a depth of 2 or 3 in., was dry enough to avoid any puddling, and the principle was to delay spring cultivations as long as the state of growth of the crop permitted. In the case of exps. Heavy VII and Heavy VIII, the date selected was so late that the crop had begun to shoot, and it was felt that further delay would have done serious harm.

As is usual with experiments, the yields shown in Table I were slightly higher than those actually obtained for the fields in which the experiments were situated; the discrepancies were small with grain, and larger with straw because a shorter stubble was left on the plots than on the rest of the field.

Some of the crops concerned were heavy, and in six cases a portion of the crop was lodged; in no instance was there any evidence that rolling tended to prevent lodging. In only one case (Light II), was harrowing found to influence lodging, and there it was increased; the percentage of the crop which was lodged on each plot was estimated, and harrowing showed a significant increase. It will also be observed that, on this occasion, harrowing increased the weight of straw significantly. Exp. Light I also suffered from an attack of "foot-rot" and the percentage infestation was estimated for each plot separately; the estimates were undoubtedly subject to a considerable error, but the figures showed that harrowing had a definite effect in controlling the disease. This observation supports Eriksson's<sup>(2)</sup> recommendation that harrowing should be used as a control measure against some species of the fungi which may be responsible for "foot-rot".

The conditions when spring cultivations are carried out must have a determining effect on their efficacy, and therefore a brief descriptive paragraph will now be devoted to each experiment.

### *Light I.*

The spring cultivations were superimposed on seed-bed preparations—plough *v.* cultivator after potatoes<sup>(3)</sup>—which produced appreciable differences in the surface; there was a fair amount of clod on the surface of the ploughed plots, whilst the autumn-cultivated plots were smoother and firmer with some slight crust on the surface. The spring cultivations were carried out under excellent conditions, the surface being dry and

## 452 *On the Spring Cultivation of Autumn-sown Wheat*

the top few inches not being so wet as to endanger texture in the hoof marks. The appropriate plots were rolled twice with the ring roll, and then harrowed twice with light seed harrows. The experimental area was fairly clean, but the harrows pulled out a little *Veronica* and some wheat; counts made 15 days later indicated that the harrow tines up-rooted between 5 and 10 per cent of the wheat plants.

### *Light II.*

The conditions for this experiment were very similar to the above, except that the surface soil was more "puffy" from winter frost and carried a considerable quantity of *Stellaria* and *Veronica*. On this occasion the ring roll and the light harrow were used only once, the harrow following the roll on the plots receiving both treatments. The work was well done and counts made subsequently indicated that the harrows removed about 3 per cent of the wheat plants.

### *Light III.*

The seed-bed enquiry incorporated in this experiment produced two very different tilths; the plots which were ploughed in autumn remained rough and loose to the tread throughout the winter, whereas those that were not were very firm. Spring cultivations had a very obvious effect on the ploughed plots, but made very little impression on the others. In this case both implements traversed their respective plots twice, and subsequent counts failed to detect any loss of plants from harrowing.

### *Heavy IV.*

Spring cultivations were tested under very good conditions in this experiment, because winter frosts had lifted the surface of the plots, which at that time were firm and dry. The ring roll and the light harrows were again used twice, and the latter removed some weeds and self-sown beans, but very little wheat.

### *Heavy V.*

This was a particularly interesting experiment as the wheat followed a bastard fallow and the field was so loose to the tread at the time the cultivations were done, that one sank up to the ankles when walking across the field. In fact this field was selected for the experiment because of its condition, which very definitely demanded the roll according to traditional views. It was fortunate that a spell of brilliant weather enabled the cultivations to be carried out under ideal conditions. In this case rolling and harrowing were both carried out twice; a two-horse

harrow was used but as the field was clean this removed very little weed.

#### *Heavy VI.*

This experiment incorporated a comparison between "gyrotilling" and normal treatment in the previous summer, and the former treatment produced plots which were very loose at the time the spring cultivations were carried out; on these latter plots horses sank in at least to the top of their hoofs, though the land was dry enough to avoid any damage to the texture. The ring roll was used twice and a two-horse harrow once, the latter pulling out some wheat; there were very few weeds.

#### *Heavy VII.*

Here the soil was dry on the surface and rather moist below, but despite the latter condition further delay was felt to be impractical, because of the advanced state of growth of the wheat.

Whilst the conditions may not have been so ideal as in the other experiments, they might still be described as quite up to the average, the remainder of the field having been similarly treated on the previous day. The ring roll and the light harrow both traversed the plots twice; the harrow removed a certain amount of wheat which on examination proved to be principally "flag". The site selected for the plots was not so clean as the site of the other experiments, the chief weed being black grass (*Alopecurus agrestis*), little of which was removed by the harrows.

#### *Heavy VIII.*

This field was selected with a definite purpose in view. The winter had been wet and cold and the surface had run together, subsequently cracking on drying; it was moreover slightly "puffy". The ring roll was used twice and reduced the surface to a fine powder. The light harrow was used once, and although a fair amount of corn buttercup (*Ranunculus acris*) was present very little was removed; on the other hand an appreciable amount of wheat was pulled out.

It may be claimed that in all these experiments the spring cultivations were conducted in accordance with the dictates of established practice. In reality, more than eight different conditions were involved, for in five of the experiments the work was coupled with enquiries into seed-bed preparation; these latter may be disregarded in the present paper, because in no case did they interact significantly with spring cultivations.

## HARVEST RESULTS

The harvest results are shown, in relative form, in Tables II-V; it will be noticed that Tables II and III are derived from samples, whereas Tables IV and V are from the whole plots.

Table II. *Effect of spring cultivations on number of ears at harvest (from samples)*

Exp.	No. of ears: general mean†	(2) (3) (4) (5) Relative no. of ears				R. + R.H. O. + H. %	H. + R.H. O. + R. %	Standard error†	Significance
		R.	H.	R.H.	O.				
Light I	17.1	—	—	102.7	100.0	—	—	2.64	Insignificant
Light II	22.4	97.1	95.8	100.1	100.0	100.7	99.4	3.40	Insignificant
Light III	14.8	88.6	96.2	99.5	100.0	95.9	103.8	2.87	Interaction significant*
Heavy IV	12.8	—	—	95.8	100.0	—	—	4.46	Insignificant
Heavy V	20.2	98.6	95.8	98.9	100.0	100.8	98.0	2.81	Insignificant
Heavy VI	19.4	107.6	109.9	105.5	100.0	101.5	103.8	2.27	Negative Interaction of R.H.*
Heavy VII	19.5	101.0	98.5	101.9	100.0	102.2	99.7	2.74	Insignificant
Heavy VIII	19.9	101.4	91.0	92.2	100.0	101.4	90.9	2.76	H. < O.**

† Mean no. of ears per foot length of drill row.

† Standard error of relative means in cols. 2-5.

\* Significant ( $P < 0.05$ ). \*\* Significant ( $P < 0.01$ ).

R. = roll. H. = harrow. R.H. = roll and harrow. O. = neither roll nor harrow.

Table III. *Effect of spring cultivations on size of ear (weight of grain corrected for number of ears) (from samples)*

Exp.	(1) (2) (3) (4) Relative no. of ears				R. + R.H. O. + H. %	H. + R.H. O. + R. %	Standard error†	Significance
	R.	H.	R.H.	O.				
Light I	—	—	108.3	100.0	—	—	4.40	Insignificant
Light II	97.9	99.4	98.4	100.0	98.5	99.9	2.86	Insignificant
Light III	104.9	102.7	96.6	100.0	99.4	97.3	4.30	Insignificant
Heavy IV	—	—	101.8	100.0	—	—	1.56	Insignificant
Heavy V	101.6	106.2	105.5	100.0	100.5	105.0	1.67	H. > O.**
Heavy VI	101.9	106.0	106.0	100.0	100.9	105.1	1.55	H. > O.**
Heavy VII	100.4	99.0	99.7	100.0	100.5	99.2	1.36	Insignificant
Heavy VIII	102.0	106.6	107.2	100.0	101.2	105.9	1.86	H. > O.*

† Standard error of relative means in cols. 1-4.

\*\* Significant ( $P < 0.01$ ).

\* Significant ( $P < 0.05$ ).

There was considerable variation in the thickness of these crops, the number of ears per foot of row varying between 12.8 and 22.4. In only one case was there a straight and significant effect of spring cultivations on number of ears; this was in the last experiment where harrowing

reduced the ear number by 10 per cent, presumably owing to the thinning of the plant. The two significant interactions are difficult to explain. In exp. Light III, both cultivations tended to reduce the number of ears when used separately, but had no effect together; in exp. Heavy VI each cultivation tended to raise ear number, though with both together the increase was less than with either. It appears, therefore, that there are cases in which rolling and harrowing may separately affect ear number in either direction, but that their tendency is to nullify each other.

It must be emphasized that the figures shown in Table III are not obtained by dividing weights of grain by ear numbers, but through the statistical method of covariance; this in effect estimates the relative weight per ear after making allowance, by means of a linear regression, for variations in ear number. In five experiments spring cultivations had no effect on ear size, but in the other three harrowing significantly raised ear weight by 5 per cent. It is interesting to observe that spring applications of nitrogen have a similar effect (4).

Table IV. *Effect of spring cultivations on yield of grain*  
(from plots)

Exp.	(1) (2) (3) (4) Relative weight of grain				R. + R.H. O. + H.	%	H. + R.H. O. + R.	%	Standard error†	Significance
	R.	H.	R.H.	O.						
Light I	—	—	110.3	100.0	—	—	—	—	3.63	Insignificant
Light II	102.0	100.1	100.5	100.0	101.2	99.3	99.3	2.51	Insignificant	
Light III	98.0	102.2	94.1	100.0	95.0	99.1	99.1	5.13	Insignificant	
Heavy IV	—	—	110.3	100.0	—	—	—	2.38	R.H. > O.**	
Heavy V	102.6	103.1	101.8	100.0	100.6	101.2	101.2	1.29	Insignificant	
Heavy VI	102.6	100.9	101.6	100.0	101.7	100.0	100.0	1.43	Insignificant	
Heavy VII	104.2	99.5	100.9	100.0	102.8	98.2	98.2	2.09	Insignificant	
Heavy VIII	100.6	102.8	103.8	100.0	100.8	102.9	102.9	1.08	H. > O.*	

† Standard error of relative means in cols. 1-4.

\*\* Significant ( $P < 0.01$ ).

\* Significant ( $P < 0.05$ ).

It would be expected that experimental errors should be lower with figures derived from the whole plots than with those derived from samples, and this was the general experience. It may be seen from Tables IV and V that the adoption of harvesting by sickle has led to a very appreciable reduction in error, the only exception being Light III, which was situated on a very patchy field. Rolling and harrowing have had very little effect on yield of grain. In exp. Heavy IV, they raised the yield by 10 per cent, and it is unfortunate that the two operations were not tested separately in that instance. In exp. Heavy VIII, harrowing raised the yield by nearly 3 per cent and, the experiment being a



## 456 *On the Spring Cultivation of Autumn-sown Wheat*

very precise one, this was significant. At first sight it would appear that the results for this experiment, shown in Tables II and III, are inconsistent with those shown for the same experiment in Table IV; this, however, is not so. From the fact that harrowing reduced ear number it might be expected that ear weight would be increased, on the basis of competition; Table III shows that harrowing had a specific effect in increasing ear weight over and above the effect of competition. Thus the actual ears on the harrowed plots were more than 5.9 per cent heavier than those on the not-harrowed plots, giving a slight advantage in yield for the harrowed plots—an advantage which was significant with the lower errors given by the produce of the whole plots.

Table V. *Effect of spring cultivations on yield of straw*  
(from plots)

Exp.	(1) (2) (3) (4) Relative weight of straw				R. + R.H. O. + H.	%	H. + R.H. O. + R.	%	Standard error†	Significance
	R.	H.	R.H.	O.						
Light I	—	—	105.3	100.0	—	—	—	—	1.94	Insignificant
Light II	101.5	105.4	113.5	100.0	104.7	—	108.6	—	3.98	H. > O.*
Light III	91.1	100.8	91.9	100.0	91.2	—	100.8	—	5.79	Insignificant
Heavy IV	—	—	105.9	100.0	—	—	—	—	3.58	Insignificant
Heavy V	101.5	97.0	97.8	100.0	101.2	—	96.6	—	1.70	Insignificant
Heavy VI	103.4	99.1	97.6	100.0	101.0	—	96.7	—	1.80	Insignificant
Heavy VII	97.2	96.2	99.1	100.0	100.0	—	99.1	—	2.62	Insignificant
Heavy VIII	103.4	98.3	101.2	100.0	103.4	—	98.1	—	1.71	Insignificant

† Standard error of relative means in cols. 1-4.

\* Significant ( $P < 0.05$ ).

Spring cultivations have had very little effect on yield of straw, the differences being significant in only one case, where harrowing raised the yield by 8.6 per cent.

These experiments have failed to show any effect of rolling on yield. The implement has been tested under practical conditions, and in some cases the soil was so loose that traditional practice demanded rolling; yet it has apparently produced no effect. On the other hand, a few definite results have been obtained with the harrow. The thinning effect has been, in some cases, quite appreciable, and on one occasion this led to a definite reduction in the number of ears at harvest. In spite of this thinning, harrowing has not decreased yield; indeed, in one case yield was definitely increased and it appears that harrowing has a decided tendency to raise weight of grain per ear. This is the same type of effect as produced by a late dressing of nitrogenous fertilizer. It is only possible to conjecture whether this apparent similarity is fortuitous, but two suggested explanations may be offered. The removal of some plants and

tillers would apparently mean that those surviving would each receive a larger share of the available nitrogen. Smith<sup>(5)</sup> has produced some evidence that excision of side tillers (in his case at a later stage of growth) increases the weight of grain per ear. On the other hand the explanation may be that the loosening of the surface provides better conditions for nitrification. The effect of nitrogenous fertilizers on yield of straw has been shown to depend on the time of application; early dressings definitely increase it, whilst late dressings exert no influence. The date at which spring cultivations are normally carried out is on the border line between these two conditions, and in only one of the present experiments was yield of straw definitely increased. It should, perhaps, be explained that the experiments provided no real test of the harrow as a controller of weeds, most of the fields being rather above the average in cleanliness.

Finally, it must be realized that the results apply only to the two types of soil described. Further series of experiments are already in progress on other soil types; these will be reported on in due course, but the present indication is that more positive results may be expected from the roll on a light chalky soil.

#### SUMMARY

Experiments have been conducted on heavy gault clay and on a gravelly loam to determine the effects of rolling and harrowing autumn-sown wheat in spring. It is concluded that rolling has no effect on yield on such soil types. In general, harrowing also had little effect on yield, although on three occasions it significantly increased the weight of grain per ear in excess of the increment to be expected from its effect on ear number.

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# THE DIGESTION OF HUSKLESS OATS BY POULTRY

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As a result of digestibility trials Halnan<sup>(1)</sup> has concluded that the suitability of varieties of oats for poultry feeding is linked with the fibre content, and that varieties with a low husk content are most suitable for this purpose. In each of three varieties investigated by this worker the fibre content proved to be wholly indigestible. Results obtained by Kaupp & Ivey<sup>(2)</sup> in America lend support to the belief that the fibre of oats is not easily digested by poultry, although a small but significant degree of digestibility was found in all cases.

There has recently been placed on the market a variety of huskless oats which differs from ordinary varieties in that it has thin, paper-like husks which are shed on threshing. In consequence, the threshed grain has an exceptionally low crude fibre content. In a note issued by the National Institute of Agricultural Botany, Biffen<sup>(3)</sup> points out that huskless varieties of oats have been known since early times, and that several have been tried out during the past half-century. Such trials have failed to provide evidence that huskless oats are capable of yielding as well as the more common varieties. Reference is made to the work of Koernicke who found that even when 25 per cent of the weight of the grain is deducted to allow for the weight of the husks of the ordinary oats, the huskless oats compared very unfavourably with them. The particular huskless variety used for the present investigation was grown at the Northumberland County Experimental Station, Cockle Park, in 1936 and yielded  $11\frac{1}{4}$  cwt. of grain and 32 cwt. of straw per acre, against means of 28 and 33 cwt., respectively, for six established varieties.

It appears therefore that at the present stage the commercial possibilities of huskless oats are somewhat uncertain. If it can be shown that they are capable of yielding as well, or nearly as well, as other varieties, or if they can be bred to do so, it seems possible that a new and valuable food for poultry will become available. Under the circumstances, and in view of the probability that the digestibility of other constituents may be affected by the exceptionally low fibre content of the variety, it was thought desirable that these oats should be made the

subject of a feeding trial with poultry. In order to allow of a comparison with a husked variety of relatively high fibre content, it was decided to carry out a preliminary trial with Victory oats, grown at Cockle Park in 1936 on a plot adjacent to that carrying the huskless oats.

### EXPERIMENTAL

Two Black Leghorn bantam cockerels were used for the purpose of this investigation, being housed in wire cages which were supported on short-legged wooden frames. The daily rations for the full duration of the trial were weighed out immediately prior to the commencement, and stored in kali bottles. At the same time, a representative sample was taken for analytical purposes and ground in a power mill.

The birds were fed twice daily, at 9.30 a.m. and 4.30 p.m., a sufficient amount of grain being given to satisfy them; this amount was determined during a pre-experimental feeding period. Twenty minutes after each feed, such grain as remained in the feeding boxes, together with any which had been spilt, was carefully collected and bottled; this material was weighed at the end of the trial, and the actual amount of grain consumed found by difference.

The excreta, which were collected on sheets of heavy plate glass placed beneath the cages, were removed each morning before feeding, transferred to wide-mouthed glass jars with ground-in stoppers, and stored in an ice box. A spatula and stiff wire brush were used for cleaning the glass plates and cage-bottoms, the birds being removed to cages similar to those normally occupied during the operation.

The trial periods were of twelve days duration, and followed on a preliminary period of four or five days. The collected excreta from each bird were weighed and mixed in a mortar every four days, weighed portions being taken for the determination of dry-matter, total nitrogen, uric acid and ammoniacal nitrogen. Total nitrogen was determined by Kjeldahl, uric acid by Woodman's<sup>(4)</sup> method, and ammoniacal nitrogen by the steam distillation method of Foreman<sup>(5)</sup>, the alcoholic extract for the last determination being prepared as described by Halnan<sup>(6)</sup>. Residual excreta not used for the above determinations were dried, milled and stored. At the end of the trial, the residual excreta from each bird were bulked and used for the determination of ether extract, fibre and ash, by the usual analytical methods.

The essential data for the determination of digestibility coefficients were obtained from the experimental results by the use of Katayama's equations<sup>(7)</sup>.

## RESULTS

*Composition of Victory and Huskless oats*

	Victory %	Huskless %
Moisture	14.90	15.46
Ether extract	4.92	4.57
*Crude protein	7.87	11.96
Nitrogen-free extract	59.90	64.68
Fibre	9.02	1.63
Ash	3.39	1.70
	100.00	100.00

\* Including:  
 True protein 6.95 9.41  
 Digestible crude protein (Wedemeyer) 7.00 10.99

*Digestibility of Victory oats*

	Oats consumed during trial (g.)						
Bird A	570.4						
Bird B	659.4						

	Composition of excreta in g.							
	Dry matter	Ash	Organic matter	Total nitrogen	Uric acid	Ammoniacal nitrogen	Ether extract	Fibre
Bird A	179.10	19.69	159.41	8.21	4.07	1.86	4.39	46.57
Bird B	214.57	19.06	195.51	10.86	5.44	2.26	6.61	54.72

## Composition of dung in g. (calculated)

	Organic matter	Crude protein	Ether extract	Fibre	N-free extract
Bird A	137.34	9.00	4.00	46.57	77.77
Bird B	166.92	13.06	6.10	54.72	93.04

*Digestibility coefficients of Victory oats*

	Organic matter	Crude protein	Ether extract	Fibre	N-free extract
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Bird A. Weight in g.					
Consumed	466.07	44.89	28.06	51.45	341.67
Undigested	137.34	9.00	4.00	46.57	77.77
Digested	328.73	35.89	24.06	4.88	263.90
Digestibility coefficient %	70.5	80.0	85.7	9.5	77.2

Bird B. Weight in g.					
Consumed	538.79	51.89	32.44	59.48	394.98
Undigested	166.92	13.06	6.10	54.72	93.04
Digested	371.87	38.83	26.34	4.76	301.94
Digestibility coefficient %	69.0	74.8	81.2	8.0	76.4

Mean digestibility coefficient %	69.8	77.4	83.5	8.8	76.8
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*Digestibility of huskless oats*

				Oats consumed during trial (g.)				
			Bird A		653.5			
			Bird B		473.7			
	Composition of excreta in g.							
	Dry matter	Ash	Organic matter	Total nitrogen	Uric acid nitrogen	Ammoniacal nitrogen	Ether extract	Fibre
Bird A	118.62	12.39	106.23	11.79	6.68	1.75	11.78	10.48
Bird B	87.23	9.09	78.14	9.06	5.22	1.29	8.57	7.84

	Composition of dung in g. (calculated)				
	Organic matter	Crude protein	Ether extract	Fibre	N-free extract
Bird A	74.90	13.62	11.22	10.48	39.58
Bird B	53.95	10.25	8.14	7.84	27.72

*Digestibility coefficients of huskless oats*

	Organic matter	Crude protein	Ether extract	Fibre	N-free extract
Bird A. Weight in g.					
Consumed	541.36	78.16	29.87	10.65	422.68
Undigested	74.90	13.62	11.22	10.48	39.58
Digested	466.46	64.54	18.65	0.17	383.10
Digestibility coefficient %	86.2	82.6	62.4	1.6	90.6
Bird B. Weight in g.					
Consumed	392.41	56.65	21.65	7.72	306.39
Undigested	53.95	10.25	8.14	7.84	27.72
Digested	338.46	46.40	13.51	-0.12	278.67
Digestibility coefficient %	86.2	81.9	62.4	—	91.0
Mean digestibility coefficient %	86.2	82.2	62.4	—	90.8

## DISCUSSION

It will be noted at the outset that on analysis the two varieties of oats tested showed wide differences in the amounts of certain constituents present. As was to be expected, the crude fibre content of the huskless oats was low, amounting only to 1.63 per cent, which is less than 20 per cent of that of the control variety. Of the remaining constituents, crude protein, N-free extract, and ash showed the most marked differences, the first of these being over 50 per cent higher in the huskless oats. This variety is also richer in N-free extract, although the difference in respect of this constituent is less marked than in the case of crude protein; on the other hand, the ash content is relatively low.

The experimental results tabulated above show that the fibre of

huskless oats is wholly undigested, while the control variety has a mean digestibility coefficient of 8.8 per cent. This difference, which is doubtless significant, can possibly be explained on the grounds that the higher N-free extract content of the huskless oats, by providing a greater supply of easily soluble carbohydrates, depresses bacterial fermentation, and so reduces the digestibility of the fibre. As a result of digestibility trials with three common varieties of oats, Halnan<sup>(1)</sup> found that in every case the fibre was wholly undigested. As already shown, the fibre content of Victory, a variety comparable with those used by Halnan, was digested to an appreciable extent; the digestibility coefficient was nevertheless much lower than that obtained by Mangold<sup>(8)</sup> and his collaborators at the College of Agriculture, Berlin. According to this worker, the view that fibre is not digested by poultry has resulted from the use of a faulty technique.

The digestibility coefficients of the crude protein in the two varieties used, viz. 82.2 and 77.4 per cent for huskless and Victory respectively, were substantially lower than the corresponding values obtained *in vitro*, but both methods show the huskless oats to have a higher digestibility than Victory. Mertins<sup>(9)</sup> has shown that with ruminants an increase in the fibre content of the ration results in reduced protein digestibility, and it is probable that the difference observed in the present trial with poultry has resulted from the same cause.

It has been stated by Halnan<sup>(1)</sup> that "the digestibility of the organic matter and N-free extract is clearly affected by the fibre content of the oat, the presence of fibre in the oat having a depressant effect on the digestibility of these two nutrients". This effect is clearly shown by the results under discussion, the Victory oats having digestibility coefficients for N-free extract and organic matter, which are less than those appertaining to huskless oats by 14.0 and 16.4 per cent respectively.

The digestibility coefficient obtained for the ether extract of the Victory oats (83.5 per cent) does not differ appreciably from coefficients obtained by other workers on commonly grown varieties. On the other hand, the digestibility of the ether extract of the huskless oats is markedly lower, amounting to only 62.4 per cent. In view of the good agreement between duplicates there can be little doubt that the difference between the coefficients is a highly significant one. The authors can offer no explanation for the comparatively low digestibility of the ether extract in huskless oats.

It has already been shown that the digestibility coefficients for huskless oats differ materially from those found for Victory, the control

variety used. As will be seen from Table I the coefficients for Victory oats are comparable with those obtained by several other workers for varieties in common use.

Table I. *Digestibility coefficients*

	Organic matter %	Crude protein %	Ether extract %	Fibre %	N-free extract %
Victory oats (Moon and Thomas)	69.8	77.4	83.5	8.8	76.8
Means for common oat varieties:					
Halnan (1)	62.4	65.8	82.8	Nil	70.6
Brown (10)	64.7	73.4	81.5	8.2	70.8
Grost (10)	—	62.3	84.0	0.5	60.8
Bartlett (11)	62.7	71.3	87.9	—	90.1
Huskless oats (Moon and Thomas)	86.2	82.2	62.4	Nil	90.8
Means for wheat:					
Halnan (12)	86.2	88.0	45.8	3.9	86.9
Bartlett (11)	82.3	75.1	53.0	—	87.0

The calculated starch equivalents for Victory and huskless oats prove to be 58.1 and 70.2 respectively. It is therefore evident that, as a feeding stuff, huskless oats are hardly in the same category as the oat varieties now commonly cultivated. From an examination of Halnan's and Bartlett's figures (cf. Table I) it appears that, in respect of digestibility, huskless oats bear a closer resemblance to wheat. Furthermore, these two cereals have very similar starch equivalents.

It is evident that in the event of huskless oats becoming available for feeding in considerable quantities, a serious error may arise in the calculation of rations if recourse is had to the values now applicable to the common varieties.

#### SUMMARY

The digestibility for poultry of a variety of huskless oats recently placed on the market has been investigated, and a comparison made with a widely grown variety of more usual fibre content.

It was shown that the fibre of the huskless oats was completely undigested, while that of the control variety had a digestibility coefficient of 8.8 per cent.

The digestibility of the crude protein in huskless oats was higher than in the variety Victory. It is suggested that this difference has resulted from the same cause as that which operates in ruminants, whereby fibre has a depressant effect on protein digestibility.

Both the organic matter and N-free extract were digested to a greater



extent in the huskless oats than in the control variety. This is in keeping with the findings of other workers on the relation between fibre content and the digestibility of these constituents.

Ether extract was digested to a markedly lower extent in the huskless oats than in Victory, which was shown to have a coefficient comparable with the values found for such other oat varieties as have been investigated. No explanation of this difference is offered.

The starch equivalent of the huskless oats proved to be substantially higher than that of Victory; indeed the former variety has been shown to bear a closer resemblance to wheat, in respect of starch equivalent and digestibility, than to any of the common oat varieties.

It is suggested that if huskless oats can be grown successfully on a commercial scale, a new and valuable feeding stuff will become available.

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# THE COMPOSITION AND DIGESTIBILITY, WHEN FED TO PIGS, OF THREE GRADES OF MEAT MEAL OF WIDELY DIFFERING FAT CONTENT

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## INTRODUCTION

DURING the carrying out of pig-feeding trials with rations containing meat meals of widely differing fat content, it was found necessary to secure information about the composition and digestibility of the three grades of meat meal under consideration. Since little or no work appears to have been done in this country on the extent to which meat meal can be utilized by pigs, it was felt that a short account of the results of these digestion trials would prove useful to those pig-feeders who favour the employment of meat meal instead of fish meal as a source of protein in the feeding of bacon pigs.

The same consignment of raw material was used in the production of the three grades of meat meal. The writers are indebted to Messrs W. Weddel and Company, Ltd., for the following details of manufacture. The raw material is trimmed off the carcasses within 1-2 hours of slaughter and is passed direct into steam-jacketed melters, except where, if necessary, it is first thoroughly washed. The material remains in the cookers until the temperature advances to about 245° F., exhaust steam at a pressure of 50-60 lb. being the source of heat. The time of cooking varies with the nature and condition of the material, but the process is continued until the product is cooked and the moisture content has been reduced to 5-8 per cent. This usually takes from 4 to 5 hours.

It will be noted that the steam does not come into contact with the material and that the process resembles that of baking in an oven. The mass is stirred during the whole process in order to facilitate the penetration of heat and the evaporation of moisture. It should be pointed out that approximately 10 per cent of bone is included with the original raw meat material to ensure better cooking in the steam-jacketed melters.

When the cooking process is complete, the surplus fat is allowed to

## 466 *The Composition and Digestibility of Meat Meal*

drain off. It was from the product remaining at this stage that the grade A meat meal (18.82 per cent of fat on the basis of dry matter) was obtained for the purpose of the present trials.

In the customary process of manufacture, however, the residual material is next pressed in an expeller to remove a further substantial portion of fat. By this means a product containing about 9 per cent of fat is obtained, and this, after milling and bagging, is ready for sale to the stock-feeder. The grade B meat meal of the present investigation (11.08 per cent of fat on the basis of dry matter) was prepared in this way.

In order to obtain the grade C meat meal (3.17 per cent of fat on the basis of dry matter), a large bulk of the grade B product was submitted to a process of "de-greasing" at 300° F. by means of petroleum benzine, super-heated steam being used in the final stages to ensure the complete removal of any residual traces of solvent.

### PLAN OF DIGESTION TRIALS

Two pure-bred Large White hogs, weighing 152 and 157 lb. respectively at the commencement of the investigation, were employed in the digestion trials, the animals having been "wormed" some 3 weeks before being brought into experiment. In the first period, the digestibility of a ration composed of barley meal, weatings and grade B meat meal was determined. The second period was devoted to measuring the digestibility of a ration consisting of barley meal, weatings and grade C meat meal, while in the third period the grade C meat meal was replaced by the grade A meat meal. In the final period, the determination of the digestibility of the basal ration of equal parts of barley meal and weatings was carried out.

The harness and metabolism cages which were used to make possible the separate collection of urine and faeces, together with the general procedure adopted in digestion trials with pigs, have been described in a previous publication (1). It is merely necessary to state here that the whole trial lasted from 21 April to 18 July 1936; that the experimental periods, during which the excreta were collected for analysis, were of 10 days' duration, and that each experimental period was preceded by a preliminary feeding period of 12 days, during which the animals were allowed to accustom themselves to the ration to be tested. The food was given in the form of a thick slop in two feeds per day, the size of the experimental rations in the different periods being adjusted to the changing live weights of the animals. The experiments ran smoothly, and the

faeces from both animals were of a normal consistency throughout. The details of the experimental rations are shown in Table I.

Table I. *Details of digestion rations*

	Period 1		Period 2		Period 3		Period 4	
	Amount	Dry	Amount	Dry	Amount	Dry	Amount	Dry
	per	matter	per	matter	per	matter	per	matter
	day	per	day	per	day	per	day	per
	g.	g.	g.	g.	g.	g.	g.	g.
Barley meal	675	592.0	787.5	689.9	900	791.4	1350	1181.5
Weatings	675	589.7	787.5	686.3	900	788.9	1350	1178.1
Grade A meat meal	—	—	—	—	600	557.0	—	—
Grade B meat meal	45.9	414.5	—	—	—	—	—	—
Grade C meat meal	—	—	525.0	488.1	—	—	—	—
	lb.		lb.		lb.		lb.	
Mean live weight	Pig 1 162		190.5		220		249.5	
of pigs during	Pig 2 166		196		224.5		254	
periods								

## COMPOSITION OF THE THREE GRADES OF MEAT MEAL

The composition of the three grades of meat meal, and of the barley meal and weatings constituting the basal diet, is shown in Table II.

Table II. *Composition of feeding stuffs (on basis of dry matter)*

	Grade A	Grade B	Grade C	Barley	Weatings
	meat meal	meat meal	meat meal	meal	
	%	%	%	%	%
Crude protein*	66.38	71.60	71.69	12.98	18.97
Ether extract	18.82	11.08	3.17	2.84	5.20
N-free extractives	2.84	2.44	4.28	76.74	68.30
Crude fibre	—	—	—	4.86	4.54
Ash	11.96	14.88	20.86	2.58	2.99
True protein	50.42	54.34	55.32	—	—
"Amides"	15.96	17.26	16.37	—	—
Lime (CaO)	3.91	4.66	7.45	—	—
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	3.99	4.52	6.25	—	—
Chlorine (Cl <sub>2</sub> )	1.30	1.99	1.54	—	—
Potash (K <sub>2</sub> O)	0.58	0.73	0.80	—	—
Soda (Na <sub>2</sub> O)	1.50	1.92	1.69	—	—
Salt by extraction (NaCl)	2.60	3.79	3.08	—	—
Moisture content as fed	7.16	7.90	7.03		

\* Using the factor 100/16.7 for converting the nitrogen of the meat meals into crude protein. The use of the factor 6.25 gives the following values: 69.28 per cent (A); 74.75 per cent (B); 74.81 per cent (C).

It is clear from the figures in Table II that, although the three grades of meat meal were made from the same consignment of raw material, the composition of grades B and C cannot be derived from the figures for grade A by assuming that the differences were caused simply by removal

## 468 *The Composition and Digestibility of Meat Meal*

of fat. This is consequent on the difficulty of accurate sampling of such heterogeneous material when working on an industrial scale.

Apart from the wide differences in fat content, to which attention has already been called, perhaps the most interesting feature of the figures in Table II is the high percentage of non-protein nitrogenous material in the meat meals. The "amides" amount to 16-17.3 per cent of the dry matter, forming nearly one-quarter of the crude protein in the different grades. It is probable that these "amides" consist mainly of extractives such as creatine and the purine bases, and although they may have little or no direct nutritive value in pig-feeding, they are of value in so far as they contribute to flavour and palatability. It may be that this high percentage of "amides" reduces the biological value of the crude protein in meat meal as compared with the protein in white fish meal, in which the amount of "amides" is relatively small, but it is scarcely likely, in view of the high percentage of true protein in the meat meals, that any such effect would be noted when the meat meal is used in the customary amounts in the rations of bacon pigs (viz. 10 per cent in the rations of the weaners, falling to 5 per cent in the final stages of fattening).

### RESULTS OF DIGESTION TRIALS

The detailed results of the different digestion periods, including the basal period, are recorded in the appendix to this paper. In Table III are summarized the digestion coefficients (mean for two pigs) of the constituents of the basal ration and the three grades of meat meal. The digestible composition of the meat meals, on the basis of dry matter, is given in Table IV, in which the corresponding figures for white fish meal(2) are also given for purposes of comparison.

Table III. *Summary of digestion coefficients (mean for two pigs)*

	Grade A meat meal (high fat) %	Grade B meat meal (medium fat) %	Grade C meat meal (low fat) %	Basal ration of barley meal and weatings %
Dry matter	85.4	88.0	75.3	82.5
Organic matter	88.3	93.1	83.9	84.1
Crude protein	90.9	93.9	87.9	86.1
Ether extract	95.4	89.0	82.3*	71.0
N-free extractives	—	—	—	88.8
Crude fibre	—	—	—	16.6

\* Taking value for Pig 2 in this case (see Appendix).

The results in Table III bring out clearly the high digestibility of the three meat meals. In the case of the main constituent, namely, the

crude protein, the digestion coefficients range from 87.9 per cent in grade C to 93.9 per cent in grade B, these values comparing very satisfactorily with the corresponding figure for white fish meal, namely, 90.2 per cent (2). The ability of pigs to digest the fat of the meat meals with a high degree of efficiency will also be noted, the fat of grade A, constituting as much as 18.8 per cent of the dry matter of the meat meal, being digested to the extent of 95.4 per cent.

Table IV. *Digestible composition of meat meals compared with corresponding figures for white fish meal (dry matter basis)*

	Grade A meat meal (high fat)	Grade B meat meal (medium fat)	Grade C meat meal (low fat)	White fish meal
	%	%	%	%
Digestible crude protein*	60.34	67.23	63.02	63.22
Digestible ether extract	17.95	9.86	2.61	3.80
Digestible N-free extractives†	2.84	2.44	4.28	1.38
Digestible organic matter	81.13	79.53	69.91	68.40
Digestible true protein‡	44.38	49.97	46.65	58.62

\* The factor 100/16.7 has been used in the case of the meat meals and 100/16 for the white fish meal. If the latter factor be applied in the case of the meat meals, the percentages of digestible crude protein are 62.98, 70.17 and 65.78 for grades A, B and C respectively, while the corresponding percentages of digestible true protein are 46.31, 52.13 and 48.69.

† Assuming small amount of glycogen to be fully digested and assimilated.

‡ Assuming "amides" to be fully assimilated.

If the verdict be based on the digestion coefficients for the crude protein and total organic matter, it is clear that the grade of meat meal usually sold for stock-feeding, namely, grade B (medium fat), is the most digestible of the three grades tested. This conclusion is arrived at if the results for the individual pigs, as well as the mean values for the two pigs, are considered. The differences in the method of production of grade A (high fat) and grade B (medium fat) were only slight, the fat being allowed to drain away from the steam-cooked meat to give grade A, whilst further fat was removed by pressure in the production of grade B. The slightly lower digestibility of grade A, therefore, can scarcely be attributed to differences in the methods of manufacture of the meat meals, but rather to the influence of the higher fat content in causing a slight lowering of the extent to which the crude protein was digested. The depression in this case, however, was only of a minor character.

Grade C (low fat) was produced from grade B by "de-greasing" with petroleum benzine at 300° F. The results in Table III suggest that this process may result in a distinct lowering of the digestibility of the crude protein, the digestion coefficient falling from 93.9 per cent in grade B to

## 470 *The Composition and Digestibility of Meat Meal*

87.9 per cent in grade C. The corresponding fall in the case of the total organic matter was from 93.1 to 83.9 per cent.

Of the three grades of meat meal, grade C (low fat) has a mineral composition and digestible composition most nearly comparable with white fish meal. Both the grade A and grade B meat meals contain a distinctly higher percentage of digestible organic matter than white fish meal. In making these comparisons, however, it should be kept in mind that a fairly substantial proportion of the digestible organic matter of the meat meals is composed of the nitrogenous extractives found in the so-called "amide" fraction of the crude protein.

### DIGESTION AND ASSIMILATION OF FAT

The utilization of the fat of the diet may be limited by three factors: (1) the enzymatic hydrolysis of the fat may be incomplete; (2) the absorption of fatty acids from the intestine may become less complete as the diet becomes richer in fat; (3) the formation of soaps in the intestine (particularly calcium soaps, when foods rich in both lime and fat, such as the grade A meat meal, are included in the ration) may render a portion of the fatty acids unavailable. This factor, while lowering the availability of both lime and fatty acids, would occasion an apparent increase in the digestion of the food fat, since the soaps thus formed would remain undissolved during the determination of the percentage of ether extract in the faeces.

It has been suggested that a more reliable value for the digestion coefficient of fat may be obtained if the amount of undigested fat is based on the sum of the free and combined organic acids together with the neutral fat of the faeces, rather than on the result of a simple determination of the percentage of ether extract in the faeces. Determinations of the organic acids and neutral fat in the composite samples of faeces were accordingly made, the methods employed being those used by Wood & Simpson in their investigations of pathological samples of human faeces (3).

The results are shown in Table V together with the fat digestion coefficients calculated on this basis. It will be noted that the amount of neutral fat in the faeces was small in all cases, a result pointing to a very efficient enzymatic hydrolysis of the fat of the meat meals. The combined organic acids formed by far the largest fraction. The digestion coefficients tend to be distinctly lower than the values obtained by the customary method of determination. It is not thought, however, that the new values are possessed of any greater degree of reliability than the con-

ventional values, since the new procedure makes the assumption that the organic acids, free and combined, in the faeces have arisen from the fat of the diet, whereas it is possible that they may have been produced in part by the bacterial breakdown in the large intestine of fibre and carbohydrate residues.

Table V. *Comparing results of two methods for determination of digestion coefficients of the fat of the meat meals (analytical figures given on basis of dry matter)*

Period		Faeces samples					Digestion coefficients of fat	
		Organic acids		Neutral fat %	Total organic acids plus fat %	Ether extract %		
		Free %	Com-bined %					
							(a) %	(b) %
Grade A meat meal (high fat)	Pig 1	2.85	6.04	0.68	9.57	6.86	80.7	93.9
	Pig 2	2.10	5.74	0.33	8.17	6.27	88.4	97.0
Grade B meat meal (medium fat)	Pig 1	2.55	3.75	0.48	6.78	7.47	87.5	89.8
	Pig 2	1.82	4.50	0.45	6.77	7.35	87.7	88.2
Grade C meat meal (low fat)	Pig 1	1.44	3.67	0.44	5.55	5.97	51.5	64.1
	Pig 2	1.31	3.69	0.49	5.49	5.43	66.4	82.3
Basal diet	Pig 1	1.62	3.21	0.56	5.39	6.72	—	—
	Pig 2	2.23	3.13	0.55	5.91	6.74	—	—

(a) Values from new procedure.

(b) Values from conventional procedure.

### SUMMARY

The composition, both organic and inorganic, and the digestibility of three grades of meat meal of widely differing fat content have been investigated. Large White hogs were used in the digestion trials.

The methods of manufacture of the meat meals have been described. They contained, on the basis of dry matter, 18.8, 11.1 and 3.2 per cent of fat respectively.

All three grades had a very high digestibility. The digestion coefficient of the main constituent, namely, the crude protein, ranged from 87.9 per cent in grade C (low fat) to 93.9 per cent in grade B (medium fat), these values comparing satisfactorily with the corresponding value for white fish meal, namely, 90.2 per cent.

The pigs digested the fat of the meat meals very efficiently. The fat of the grade A meat meal, forming 18.8 per cent of the dry matter, was digested to the extent of 95.4 per cent.

The grade B meat meal (medium fat) was the most digestible of the three grades tested. This is the grade that is marketed for feeding to livestock. The slightly lower digestibility of grade A (high fat) is attributed



## 472 *The Composition and Digestibility of Meat Meal*

to the influence of the high fat content in causing a slight lowering of the extent to which the crude protein is digested.

The grade C meat meal (low fat) was produced from the grade B by "de-greasing" with petroleum benzine at 300° F. The results suggest that this process may result in a distinct lowering of the digestibility of the meal. The grade C meat meal is the most comparable with white fish meal in both digestible and mineral composition. In comparing the meat meals with white fish meal, however, it must be kept in mind that the former contain a much higher proportion of non-protein nitrogenous material.

The meat meals investigated in this work were specially manufactured by Messrs W. Weddel and Company, Ltd. in accordance with the specifications of the writers, who gladly take this opportunity of tendering their sincere thanks. Acknowledgements are also due to Mr A. J. Codling for considerable assistance in the carrying out of the analytical work.

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APPENDIX  
*Digestibility Tables*

	Fig 1				Fig 2			
	Dry matter g.	Organic matter g.	Crude protein g.	Ether extract g.	Dry matter g.	Organic matter g.	Crude protein g.	Ether extract g.
<b>Period 1 (Grade B meat meal)</b>								
Consumed:								
Barley meal	592.00	576.73	76.84	16.81	592.00	576.73	76.84	16.81
Weatings	589.70	572.07	111.87	30.66	589.70	572.07	111.87	30.66
Meat meal	414.50	352.83	296.78	45.93	414.50	352.83	296.78	45.93
Total	1596.20	1501.63	485.49	93.40	1596.20	1501.63	485.49	93.40
Voided	248.59	203.50	43.68	18.41	265.29	210.75	45.00	19.23
Digested	1347.61	1298.13	441.81	74.99	1330.91	1290.88	440.49	74.17
Digested from basal food	975.54	967.48	161.91	33.74	973.20	964.47	162.91	33.64
Digested from meat meal	372.07	330.65	279.90	41.25	357.71	326.41	277.58	40.53
Digestion coefficients of grade B meat meal, %	89.76	93.71	94.31	89.81	86.30	92.51	93.53	88.24
<b>Period 2 (Grade C meat meal)</b>								
Consumed:								
Barley meal	689.90	672.10	89.55	19.59	689.90	672.10	89.55	19.59
Weatings	686.30	665.78	130.19	35.69	686.30	665.78	130.19	35.69
Meat meal	488.10	386.28	349.92	15.47	488.10	386.28	349.92	15.47
Total	1864.30	1724.16	569.66	70.75	1864.30	1724.16	569.66	70.75
Voided	368.37	280.28	75.56	21.55	355.87	269.87	70.44	18.84
Digested	1496.93	1443.88	494.10	49.20	1508.43	1454.29	499.22	51.91
Digested from basal food	1136.10	1126.71	188.54	39.29	1133.39	1123.22	189.70	39.18
Digested from meat meal	359.83	317.17	305.56	9.91	375.04	331.07	309.52	12.73
Digestion coefficients of grade C meat meal, %	73.73	82.11	87.32	64.06	76.84	85.71	88.45	82.29
<b>Period 3 (Grade A meat meal)</b>								
Consumed:								
Barley meal	791.40	770.98	102.72	22.48	791.40	770.98	102.72	22.48
Weatings	788.90	765.31	149.65	41.02	788.90	765.31	149.65	41.02
Meat meal	557.00	490.39	369.74	104.83	557.00	490.39	369.74	104.83
Total	2137.30	2026.68	622.11	168.33	2137.30	2026.68	622.11	168.33
Voided	366.61	309.44	73.88	24.79	350.10	294.75	63.88	21.65
Digested	1770.69	1717.24	548.23	143.54	1787.20	1731.93	558.23	146.68
Digested from basal food	1304.59	1293.81	216.53	45.14	1301.47	1289.79	217.87	45.00
Digested from meat meal	466.10	423.43	331.70	98.40	485.73	442.14	340.36	101.68
Digestion coefficients of grade A meat meal, %	83.68	86.35	89.71	93.87	87.20	90.16	92.05	96.99
<b>Period 4 (Basal period)</b>								
Consumed:								
Barley meal	1181.50	1151.02	153.36	33.56	1181.50	1151.02	153.36	33.56
Weatings	1178.10	1142.87	223.49	61.26	1178.10	1142.87	223.49	61.26
Total	2359.60	2293.89	376.85	94.82	2359.60	2293.89	376.85	94.82
Voided	411.72	362.11	53.50	27.42	416.30	368.03	51.50	27.62
Digested	1947.88	1931.78	323.35	67.40	1943.30	1925.86	325.35	67.20
Digestion coefficients of basal ration, %	82.55	84.21	85.80	71.08	82.36	83.96	86.33	70.87

*Note.* The care of the experimental animals was in the hands of Messrs V. Thurlbourn and C. Bendall.

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# THE ESTIMATION OF LEAF AREA IN FIELD CROPS

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(With One Text-figure)

THE leaf area of a plant is a major determinant of its growth, for the new material produced by the plant in an interval of time is dependent on the size of its assimilating system. West *et al.* (4), and Gregory (2,3) have developed methods of growth analysis, using the rate of increase of dry matter per unit area of leaf ("Unit Leaf Rate" of West *et al.*; "Net Assimilation Rate" of Gregory) as a measure of the balance of the rates of assimilation and respiration.<sup>1</sup> An analysis of plant growth in terms of this function and of the changes with time in leaf area provides more fundamental information than an analysis in terms of the relative growth rate.

In pot culture experiments, particularly in the early stages of growth, it is comparatively easy to measure leaf area directly, because the uniformity of the material allows of the use of a small number of plants. In the later stages, however, when the leaves become numerous and large, measuring the leaf area of every leaf may become extremely laborious. This difficulty is very much intensified in work on field crops. The great variability of the crop necessitates that all observations be made on a number of random samples, each consisting of many plants, in order that the growth changes and the magnitude of the experimental errors may be estimated accurately. The labour of measuring the leaf area of such large samples of plants directly would be impracticably great, for it would involve measuring separately several hundreds, possibly thousands, of leaves at each sampling time.

It is easy, however, to determine the mean leaf weight per plant, by cutting off and weighing the leaves of each sample, and dividing the total leaf weight by the number of plants. A high correlation exists between leaf area and leaf weight, and this fact has been utilized by Ballard & Petrie (1), who have used leaf weight instead of leaf area in calculating unit leaf rate. They point out, however, that other workers have found a drift in the leaf area : leaf weight ratio with time during

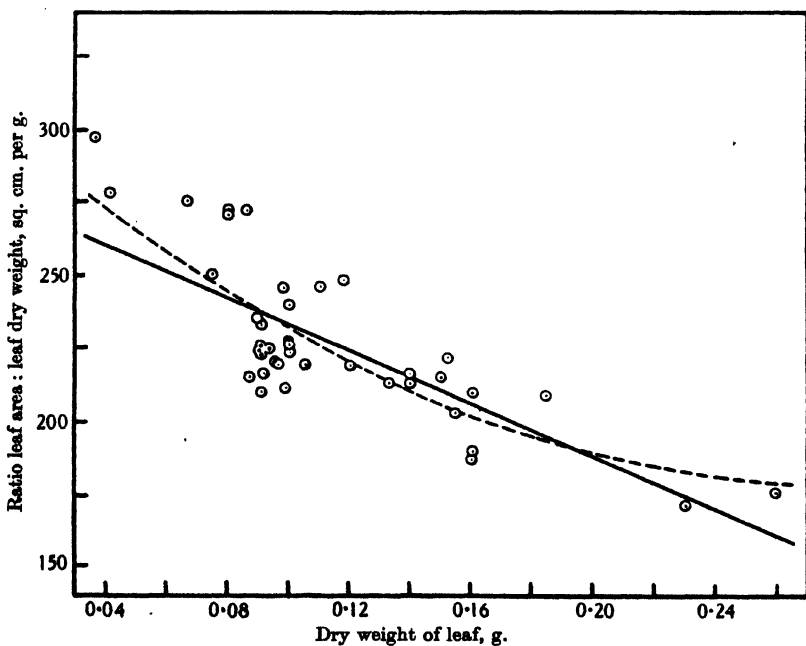
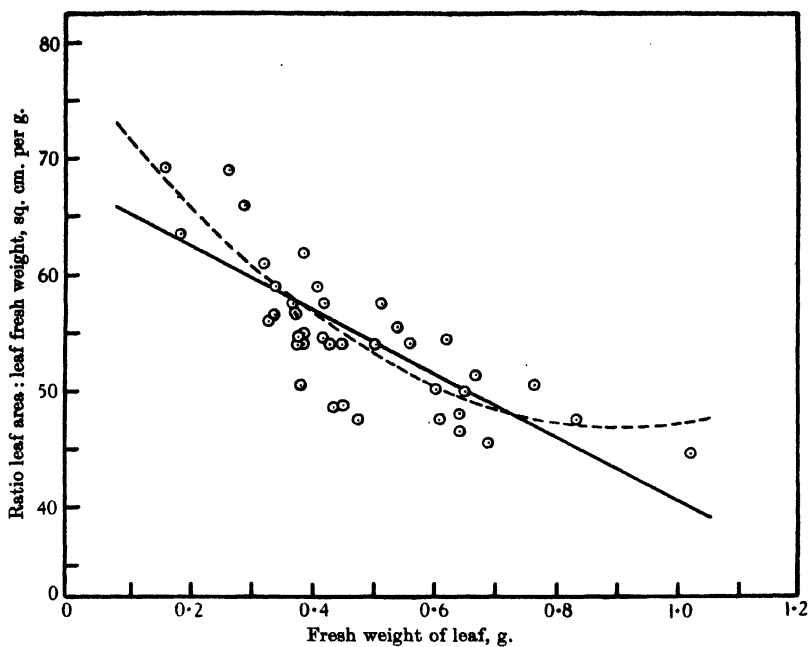


Fig. 1. Relation between leaf area : leaf weight ratio and leaf weight for single wheat leaves. Samples taken on 12th May, 1933.

the growing period, so that the relation between the unit leaf rate, as calculated by them, and as defined by West *et al.*, is not a simple one.

The correlation between leaf area and leaf weight may be made the basis of an indirect method of measuring leaf area. The procedure suggested in this paper for field crops is to estimate mean leaf weight from large random samples of plants, and to determine the leaf area : leaf weight ratio on quite small samples of single leaves. A similar method of estimating grain yield in cereal crops, by sampling for grain : straw ratio and weighing total produce has been suggested by Yates & Zacopanay (5). Gregory (3) has used an indirect method of estimating leaf area on the basis of the leaf area : leaf weight relation, in the later stages of a study of the growth of barley in pot culture, but gives no details of his procedure.

If the leaf area : leaf weight ratio were independent of leaf weight, it would be sufficient to estimate the mean leaf area per plant by multiplying the leaf area : leaf weight ratio, determined on the small sample of leaves, by the mean leaf weight per plant, obtained from the large sampling. On *a priori* grounds, however, it is obvious that the leaf area : leaf weight ratio must decrease with increasing leaf weight, for as the leaf grows in area it also increases in thickness.

Preliminary observations made on wheat leaves showed that there was, in fact, a negative regression of the leaf area : leaf weight ratio on leaf weight. The linear regression was found to account for 55 to 70 per cent of the variance of the ratio, and on fitting a second order term the additional reduction of the variance was small, ranging from 1 to 7 per cent. The nature of the relationship is shown in Fig. 1, where the leaf area per unit weight of leaf is plotted against leaf weight for forty wheat leaves, and the linear and second order regression lines are indicated. A similar type of relation holds whether fresh weight or dry weight is used, and whether single leaves or all the leaves of a shoot taken together are considered. In an experiment carried out on sugar-beet and mangolds, some of the results of which are considered later in this paper, negative linear regressions were found on all occasions, significant in 102 out of a total of 120 samplings. The mean reduction of the variance due to the regression was 71 per cent. It may be concluded that the linear regression is an adequate expression of the relationship between the leaf area : leaf weight ratio and leaf weight, and that the second order regression gives very little additional information to compensate for the large increase in the labour of computation, which fitting the extra term would involve.

METHOD OF ESTIMATING MEAN AREA PER LEAF AND  
MEAN LEAF AREA PER PLANT

Let  $A$  be the leaf area of a leaf of weight  $W$ , and let  $\bar{A}$  and  $\bar{W}$  be the mean values of  $A$  and  $W$  for the whole population of  $N$  leaves. Assuming that the relation between the leaf area : leaf weight ratio and leaf weight is linear, we have, apart from experimental errors,

$$\frac{A}{W} - \left( \frac{\bar{A}}{\bar{W}} \right) = \beta (W - \bar{W})$$

or  $\frac{A}{W} = \kappa + \beta W$ , where  $\kappa = \left( \frac{\bar{A}}{\bar{W}} \right) - \beta \bar{W}$

or  $A = \kappa W + \beta W^2$ .

Making a summation over the whole population we have

$$S(A) = \kappa S(W) + \beta S(W^2). \quad \dots\dots(1)$$

Dividing by  $N$  we have

$$\bar{A} \text{ (mean area per leaf)} = \kappa \bar{W} + \beta \bar{W}^2. \quad \dots\dots(2)$$

If the mean number of leaves per plant for the whole population is  $\bar{L}$ , then the mean leaf area per plant =  $\bar{A} \cdot \bar{L} = \bar{L} [\kappa \bar{W} + \beta \bar{W}^2]$ .  $\bar{A}$ ,  $\bar{W}$  and  $\bar{L}$  are estimated accurately from a large random sampling but not  $\bar{W}^2$ ,  $\kappa$  and  $\beta$ , since these involve the determination of individual leaf weights and areas.

Now  $S(W^2) = S(W - \bar{W})^2 + N \bar{W}^2$  and substituting in (1) we have

$$S(A) = \kappa S(W) + \beta N \bar{W}^2 + \beta S(W - \bar{W})^2.$$

Dividing by  $N$ ,

$$\bar{A} = \kappa \bar{W} + \beta \bar{W}^2 + \frac{\beta S(W - \bar{W})^2}{N}. \quad \dots\dots(3)$$

In addition to the large sample, a small subsample of  $n$  leaves is taken, and the area and weight of each leaf is determined. From these areas and weights,  $k$  and  $b$ , estimates of  $\kappa$  and  $\beta$  respectively are calculated

( $k = \left( \frac{a}{w} \right) - b\bar{w}$ , where  $a$  and  $w$  are areas and weights of leaves of the subsample).

Now it is known that  $\frac{1}{n-1} S(w - \bar{w})^2$  taken over the subsample is an unbiased estimate of  $\frac{1}{N-1} S(W - \bar{W})^2$  taken over the large sample.

Hence an unbiased estimate of  $\frac{1}{N} S(W - \bar{W})^2$  is  $\frac{N-1}{N} \cdot \frac{S(w - \bar{w})^2}{n-1}$ , and

## 478 *The Estimation of Leaf Area in Field Crops*

since  $N$  is large (in the examples which follow  $N=500$  to  $1000$ ), the factor  $\frac{N-1}{N}$  may be replaced by 1.

Substituting in (3), we have

$$A_r \text{ (estimate of } \bar{A}) = k\bar{W} + b\bar{W}^2 + b \frac{S(w-\bar{w})^2}{n-1}, \quad \dots\dots(4)$$

in which  $\bar{W}$  is determined from the large sample, and  $k$ ,  $b$  and  $\frac{S(w-\bar{w})^2}{n-1}$  are obtained from the subsample.

It is important that the subsample be a strictly random selection from the whole population, in order that  $\frac{S(w-\bar{w})^2}{n-1}$  may be an unbiased estimate of  $\frac{S(W-\bar{W})^2}{N-1}$ . If the subsample were to be used only for the estimation of the regression coefficient  $b$ , it would be preferable to select it to give the widest possible range of leaf weight so as to estimate  $b$  as accurately as possible, but this is not permissible if the subsample is also used to estimate the variance of mean leaf weight as well as  $b$ . A possible alternative method would be to take two subsamples, one selected to give a wide range of leaf weight to be used for the estimation of  $b$ , and the other, perhaps somewhat larger, a strictly random selection to provide an estimate of the variance of mean leaf weight. This method might be preferable if considerations of the work involved in measuring the leaf areas require that the subsample on which  $b$  is estimated be too small to give an accurate estimate of the variance.

It can be shown that the estimate of  $\bar{A}$  obtained by this method is unbiased. From (4) the mean value of  $A_r$  from repeated subsamples of  $n$  leaves reduces to

$$\begin{aligned} \bar{A} &= \kappa\bar{W} + \beta\bar{W}^2 + \beta\sigma^2, \text{ where } \sigma^2 \text{ is the variance of leaf weight} \\ &= \kappa\bar{W} + \beta\bar{W}^2 + \beta(\bar{W}^2 - \bar{W}^2) \\ &= \kappa\bar{W} + \beta\bar{W}^2, \end{aligned}$$

which is the true value of  $\bar{A}$  (expression (2) above).

### BIAS IN OTHER METHODS OF ESTIMATING MEAN AREA PER LEAF

The mean area per leaf might be estimated from the product of the mean leaf weight  $\bar{W}$ , obtained from the large sample, and the mean

leaf area : leaf weight ratio  $\left( \frac{S\left(\frac{a}{w}\right)}{n} \right)$  calculated from the subsample.

It is obvious that the estimate of  $\bar{A}$  obtained in this way must be positively biased, since the small leaves, which have a high leaf area : leaf weight ratio, will be overweighted, and the larger leaves, with smaller leaf area : leaf weight ratio, underweighted. The magnitude of the bias can be estimated as follows. For any leaf of the subsample of  $n$  leaves

$$\frac{a}{w} = \kappa + \beta w. \text{ Therefore } \overline{\left(\frac{a}{w}\right)} = \kappa + \beta \bar{w}$$

and the estimate of  $\bar{A} = \bar{W} \times \overline{\left(\frac{a}{w}\right)} = \bar{W} (\kappa + \beta \bar{w})$ .

The average estimate of  $\bar{A}$  from repeated subsamples of  $n$  leaves

$$\begin{aligned} &= \bar{W} (\kappa + \beta \bar{W}) \\ &= \kappa \bar{W} + \beta \bar{W}^2 - \beta \sigma^2, \text{ since } \sigma^2 = \bar{W}^2 - \bar{W}^2. \end{aligned}$$

The true value of  $\bar{A} = \kappa \bar{W} + \beta \bar{W}^2$ , so that the bias introduced is  $-\beta \sigma^2$ , which is positive since  $\beta$  is negative, and independent of the size of the subsample ( $n$ ).

The objection to this method is partly mitigated if instead of the

unweighted mean leaf area : leaf weight ratio  $\frac{S\left(\frac{a}{w}\right)}{n}$ , the weighted mean  $\frac{S(a)}{S(w)}$  is used, so that  $\bar{A}$  is estimated as  $\bar{W} \cdot \frac{S(a)}{S(w)}$ . It can be shown, however, that this estimate is also positively biased, though to a less extent than the estimate from the unweighted mean. Thus, for any leaf of the subsample of  $n$  leaves

$$a = \kappa w + \beta w^2.$$

Therefore

$$\bar{a} = \kappa \bar{w} + \beta \bar{w}^2.$$

The weighted mean leaf area : leaf weight ratio

$$\frac{S(a)}{S(w)} = \frac{\bar{a}}{\bar{w}} = \kappa + \beta \frac{\bar{w}^2}{\bar{w}},$$

and the estimate of  $\bar{A} = \bar{W} \times \frac{\bar{a}}{\bar{w}} = \kappa \bar{W} + \beta \frac{\bar{w}^2}{\bar{w}} \cdot \bar{W}$ .

The mean value of the estimate of  $\bar{A}$  from repeated subsamples of  $n$  leaves

$$\begin{aligned} &= \kappa \bar{W} + \beta \bar{W} \left[ \text{mean value of } \frac{\bar{w}^2}{\bar{w}} \right] \\ &= \kappa \bar{W} + \beta \bar{W} \left[ \text{mean value of } \bar{w} + \frac{S(w - \bar{w})^2}{n\bar{w}} \right] \quad \dots\dots(5) \end{aligned}$$



$$\begin{aligned}
 (\text{since } \frac{\bar{w}^2}{\bar{w}} &= \frac{S(w^2)}{n\bar{w}} = \frac{S(w - \bar{w})^2 + n\bar{w}^2}{n\bar{w}} = \frac{S(w - \bar{w})^2}{n\bar{w}} + \bar{w}) \\
 &= \kappa \bar{W} + \beta \bar{W} \left( \bar{W} + \frac{n-1}{n} \cdot \frac{\sigma^2}{\bar{W}} \right) \text{ approximately,} \\
 &= \kappa \bar{W} + \beta (\bar{W}^2 + \sigma^2) - \frac{1}{n} \beta \sigma^2 \\
 &+ \kappa \bar{W} + \beta \bar{W}^2 - \frac{1}{n} \beta \sigma^2.
 \end{aligned}$$

The bias introduced is therefore  $-\frac{1}{n} \beta \sigma^2$ , or  $1/n$ th of the bias in the estimate from the unweighted mean. It is dependent on the size of the subsample, becoming zero when  $n$  is very large, as obviously it must, since for the whole population  $\bar{W} \cdot \frac{\bar{A}}{\bar{W}}$  is equal to  $\bar{A}$ . It should be pointed out that these estimates of the bias are correct only if the subsample is a random selection from the whole population, so that the mean of  $\bar{w}$  tends to  $\bar{W}$  in repeated subsamples of  $n$  leaves, and the estimate of  $\sigma^2$ , the variance of mean leaf weight, is unbiased.

#### EXPERIMENTAL RESULTS

A sampling experiment was carried out on sugar-beet and mangolds in 1934, in which growth observations were made by sampling at fortnightly intervals. The experiment consisted of six blocks, each of two plots, one of sugar-beet and one of mangolds, and the blocks were sown singly at successive intervals of a fortnight. Sampling was begun when the crops were thinned and ten complete samplings were carried out after the thinning of the last sown plots. On each occasion a random sample of twenty plants was taken from each plot, the number of leaves in the whole sample was counted, and the total fresh weight of leaf lamina determined. A random subsample of ten leaves was taken, one from each of ten plants selected at random from the twenty plants of a sample. The lamina was cut off and weighed and its area measured by printing on "blue-print" paper, cutting out the print and weighing it. The mean leaf area per plant was calculated for each sample by the three methods which have been discussed. The estimates obtained by the unweighted mean leaf area: leaf weight ratio method were consistently greater than those calculated by the regression method. The estimates from the weighted mean showed a similar but smaller positive bias. The mean values for the sixty samplings of sugar-beet and of mangolds are shown in Table I.

Table I. *Mean leaf area per plant, sq. dm. (mean of sixty samplings)*

Method of estimation	Sugar-beet	Mangolds
Unweighted mean	51.41	40.89
Weighted mean	40.81	30.49
Regression	38.03	28.97
Bias:		
Unweighted mean	13.38	11.92
Weighted mean	2.78	1.52
Ratio unweighted : weighted	4.8	7.8

It has been shown theoretically that the bias in the unweighted mean estimate should be  $n$  times the bias in the weighted mean estimate, where  $n$  is the number of leaves in the subsample. In this experiment  $n=10$ , and the ratio of the biases was found to be 4.8 for sugar-beet and 7.8 for mangolds. The latter figure is in fair agreement with theory, but the discrepancy for sugar-beet is more serious. The most probable explanation is that the estimate of mean leaf weight in the subsample was positively biased. In the sugar-beet there was a considerable development of axillary buds, forming many leaves of small area. In selecting the subsample of ten leaves, these were ignored, as it was difficult to devise any simple method of strict random selection which would include them, but they were included in the estimate of mean leaf weight in the large samples ( $\bar{W}$ ). The leaves on the main axis were selected by counting back from the youngest leaf in the order of production, until a leaf of a number selected from a table of random numbers was reached. In the mangolds, however, axillary leaves were rare. If  $\bar{w}$  is an unbiased estimate of  $\bar{W}$ , it would be expected that in a series of samples, the number of occasions on which  $\bar{w}$  exceeded  $\bar{W}$  would be equal to the number on which  $\bar{w}$  was less than  $\bar{W}$ . This was found to be true approximately for the mangolds, but for sugar-beet the number of occasions on which  $\bar{W}$  was greater than  $\bar{w}$  was markedly in excess of expectation. This is shown in Table II.

Table II

	Sugar-beet	Mangolds
Number of occasions on which $\bar{w}$ was greater than $\bar{W}$	40	28
Number of occasions on which $\bar{w}$ was less than $\bar{W}$	20	32

This bias in the estimate of  $\bar{w}$  may have affected the estimates of mean leaf area by the regression method, for though  $k$  and  $b$  will not have been affected, the estimate of  $\sigma^2$  may be biased.

The estimates of mean leaf area per plant calculated by the unweighted mean method were considerably more variable than estimates

by the weighted mean, or regression methods. Table III shows the residual variances, after elimination of variance due to plots, times of sampling and the interactions with time of the linear regressions on sowing date.

Table III. *Residual variance of mean leaf area per plant (72 D.F.)*

Unweighted mean method	74.89
Weighted mean method	35.11
Regression method	48.14

The variances of the estimates calculated by the weighted mean and regression methods did not differ significantly.

A similar method of estimating mean leaf area per plant and per metre row of crop has been employed for wheat. The labour of cutting off and weighing the leaves from a large number of random samples, in order to determine the mean leaf weight  $\bar{W}$ , was found to be impracticably great, and to avoid this, the estimates were based on determinations of the leaf area : plant dry weight ratio and its linear regression on plant dry weight. The method of calculation was exactly similar to that described above, where the leaf area : leaf weight ratio and its regression on leaf weight were used. Significant negative regressions of the leaf area : plant dry weight ratio were found during the period from the beginning of May onwards, but during the earlier stages of growth the regression coefficients were small and not significant. Positive and negative values of the coefficient were equally frequent, indicating that during this period of growth the leaf area : plant dry weight ratio was independent of plant dry weight. The regression of the leaf area : leaf dry weight ratio on leaf dry weight was found to be consistently negative and significant on almost all occasions, as in the sugar-beet and mangold data.

#### SUMMARY

1. It is shown that the leaf area : leaf weight ratio decreases with increasing leaf weight.
2. The relation between the leaf area : leaf weight ratio and leaf weight is well fitted by a linear regression equation.
3. A method of estimating the mean leaf area per leaf or per plant of a field crop by means of this regression is described. The mean weight per leaf is determined by a large sampling, and the leaf area : leaf weight ratio and its regression on leaf weight are estimated on a small subsidiary sample.

4. Alternative methods of estimation from the mean leaf weight and either the unweighted or the weighted mean leaf area : leaf weight ratio are shown to give positively biased estimates of mean leaf area.

5. It is emphasized that the small sample, from which the leaf area : leaf weight ratio and its regression on leaf weight are determined, must be a strictly random selection from the whole population.

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WEANING WEIGHT OF PIGS AND LITTER  
SAMPLING WITH REFERENCE TO  
LITTER SIZE

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(With One Text-figure)

INTRODUCTION

THE first part of this paper is concerned chiefly with the relation between weaning weight and litter size. It has been maintained that the average weight per pig at weaning is independent of the size of the litter (*Bull. N.Z. Dep. sci. industr. Res.*, 1930; Blissett, 1932; McMeekan, 1936). Average weights per pig given by Wild (1927) also show little variation with litter size. As against this, Murray (1934) presented data showing a decrease in weight per pig at 8 weeks as litter size increased. From their work on Mangalitza pigs, Contescu & Roman (1935) conclude that the weight of the whole litter is not proportional to the number of pigs in it and therefore the average weight per pig cannot remain unaffected by litter size. Further, Johansson (1931) finds that at 3 weeks there is a decrease in weight per pig as litter size increases, and this fact, taken in conjunction with the high correlations observed by Axelsson (1933) and Kronacher & Hundsdorfer (1936) between weights at 3 and 8 weeks, renders it doubtful whether the relation between litter size and weaning weight can yet be expressed in simple terms which are generally applicable. In view of the existing differences of opinion, the records of the past five years of a herd of Large White pigs maintained by the Institute of Animal Genetics, Edinburgh, have been examined with respect to the point at issue.

The second part deals principally with the associated question of litter sampling for the purposes of litter testing. With the exception of some investigations by Lush (1936), which will be mentioned in more detail later, there appears to be very little information available about this matter. Although the size of sample which can be dealt with by testing stations is affected by economic considerations, it is nevertheless as well to know something of the degree to which various types of sample can

## 486      *Weaning Weight and Litter Sampling of Pigs*

represent the litter from which they are drawn, and an attempt has been made to supply some of the needed information. Underlying both these questions are fundamental problems concerning the growth of a litter, and it seems that until these have yielded to further study the selection of breeding stock at, or before, weaning must necessarily be carried out without much reference to genetic values.

### ANALYSIS OF WEANING WEIGHTS

The majority of the litters were weaned and weighed exactly 8 weeks after birth; the weights of the remainder have been subjected to the necessary small corrections to make them comparable. The records date from 1931, and the management of the sows immediately before and after farrowing, and of the young pigs, has been uniform throughout. Within a year of starting the work it was considered that litters might be affected by differential treatment of the sows prior and subsequent to conception. Accordingly, since May 1932, all the sows have been treated similarly, and have received the same feeding before, as well as after, farrowing. Apart from ensuring access to the sows' ration, no attempt has been made to encourage litters with supplementary creep feeding.

For the purposes of the analyses, the average weaning weight has been calculated for each litter from the weights of the pigs alive at weaning, and the averages have been grouped according to the number of pigs in the litter.

Since it has been shown by Johansson (1931), Kríženecký (1935), and others that the age of the sow has an influence on the weight of the litter, the average weights obtained have been further subdivided into four classes based on litter sequence. Table I gives the condensed weaning weight data.

Table I. *Average weight in pounds per pig alive at weaning (with the number of litters from which the averages were obtained)*

Litter no.	Litter size									
	4	5	6	7	8	9	10	11	12	15
1	30.55	29.96	29.08	26.02	25.81	23.36	24.42	23.1	21.8	—
	4	3	4	5	8	5	5	2	1	—
2	—	—	33.3	26.32	28.33	27.92	28.1	28.57	26.53	19.3
	—	—	2	5	4	6	4	3	3	1
3	36.8	—	—	29.5	34.0	27.8	30.47	28.53	22.3	—
	1	—	—	2	2	7	3	3	2	—
4-9	—	36.7	29.55	30.1	26.05	29.93	28.03	31.0	22.96	—
	—	1	2	4	2	7	3	1	5	—

Inspection of this table shows that the average litter weight of gilts' litters is below that of older sows. There is also a fairly well-defined difference in the average weight of large and small litters, although within the range of litter sizes 7-11 the average weight seems to remain fairly constant for sows which have had one or more litters. The averages for gilts show a gradual reduction in average weight throughout the range. On the assumption that a simple linear regression of weight on litter size exists, an analysis of the variance of these weights has been made and is given in Table II.

Table II. *Analysis of variance in litter weights*

Variance	D.F.	Sum of squares	Mean square	S.D.	Log S.D.	z
First litter						
Regression	1	205.60	205.60	14.339	2.663	0.984*
Deviations	7	22.23	3.18	1.783	0.578	-1.101†
Within classes	28	804.94	28.75	5.362	1.679	—
Total	36	1032.77	28.69	—	—	—
Second litter						
Regression	1	48.69	48.69	6.978	1.943	0.475 N.S.
Deviations	6	102.98	17.16	4.142	1.421	-0.047 N.S.
Within classes	20	376.94	18.85	4.342	1.468	—
Total	27	528.61	19.58	—	—	—
Third litter						
Regression	1	119.47	119.47	10.931	2.392	0.597 N.S.
Deviations	5	98.58	19.72	4.441	1.491	-0.304 N.S.
Within classes	13	471.67	36.28	6.023	1.795	—
Total	19	689.72	36.30	—	—	—
Fourth-ninth litters						
Regression	1	153.87	153.87	12.406	2.519	1.326*
Deviations	6	111.60	18.60	4.313	1.462	0.269 N.S.
Within classes	17	184.81	10.87	3.297	1.193	—
Total	24	450.28	18.76	—	—	—

\*  $P > 5\%$ . †  $P > 1\%$ . N.S. = non-significant.

The coefficients of regression are: (1)  $-1.111$ , (2)  $-0.637$ , (3)  $-1.333$ , (4)  $-1.216$ , and the analysis shows that the first and the last are significant. The standard errors of the other two are less than the coefficients, so that there appears to be a significant regression of weight on litter size equivalent to about 1 lb. per unit increase or decrease in litter size. It will be noted that deviations from regression are remarkably small for gilts' litters. Whether there is a biological basis for this is doubtful.

The fact that a straight regression line can be fitted to this type of data should not blind the investigator to the possibility that the relation between weaning weight and size of litter is not really linear. On the



contrary, there are *a priori* reasons for thinking that the regression line which has been fitted is misleading. The factors which are known to affect the growth of pigs up to weaning are hardly likely to interact in a simple way. The increase in milk production by the sow with increase in size of litter is not linear, nor is it probable that the change in efficiency of the pigs with varying quantities of milk is linear, so that considering these two factors alone, it is unlikely that the observed regressions are more than approximations. It may be shown, in fact, that a parabolic curve or a cubic curve may fit equally well the same data. Approximate equations involving square and cubic terms have been worked out for the data relating to the first litters, but the third and fourth terms of the equations are so small as to make it not worth while to carry this type of analysis any further with the present figures. The parabolic curve appeared to give a slightly better fit than the straight line and to be practically the same as the cubic curve. There would certainly be no significant differences among them.

#### RELATION BETWEEN GROWTH OF PIGS AND MILK SUPPLY

As a descriptive statistic the linear regression coefficient probably fits as closely as any the net result of all the factors affecting weaning weight in relation to litter size. Nevertheless, the form of the curve plotted from the average weaning weights obtained from second to ninth litters suggests an explanation of results which do not conform to the straight regression line. The changes in average weaning weight shown in Fig. 1 indicate, if they are accepted for the moment at their face value, that there is a range of litter sizes in which there are no differences in average weaning weight, and which extends on either side to extremes of litter size where the average weaning weight is greater or less than in the central part of the range. Now although the numbers of litters on which this figure is based are too small to be of much significance *per se*, the fact that they agree in part with both of the opposed views as to the effect of litter size on weaning weight suggests an explanation by which these views may be reconciled.

Starting from the observation of Bonsma & Oosthuizen (1935) and Dschapardise (1936) that the amount of milk per piglet falls off with increasing litter size although the total milk production of the sows rises, and assuming with the former that there are changes in the efficiency with which a piglet can deal with varying quantities of milk, the average weaning weight may be visualized as a function of these two variables.

It may be supposed that the amount of milk that a pig receives will vary according to the size of litter, on the grounds that increasing stimulus by suckling will not result in equal but in diminishing increments of milk, and that, when the number in a litter exceeds the number of teats, the available milk must be shared. It may also be supposed that after the maintenance requirements are satisfied the growth of a pig will be in direct proportion to the amount it receives until the quantity reaches a certain point, after which the gain in weight per unit of milk consumed over maintenance requirements will gradually fall as the quantity of milk increases. Assuming that weight at weaning is a function of these two variables, the change in average weight with change in litter size shown

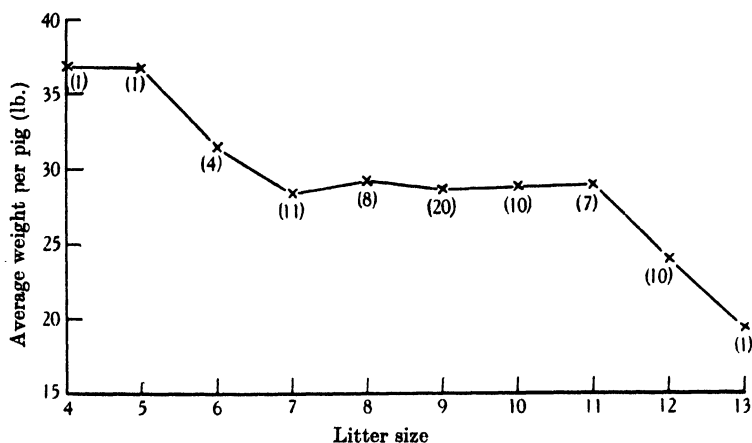


Fig. 1. Showing relation between mean weight per pig at weaning for litters of different sizes excluding gilts' litters. (Number of litters in brackets.)

in Fig. 1 may be interpreted in the following way. Over the range of 7–11 in litter size, increased economy of gain has offset any reduction in milk supply. In litters larger than 12 this did not happen, and the average weight decreased. In litters smaller than 7 the reduction in economy of gain is more than offset by the rapidly increasing quantity of milk and the average weight rises. Hypothetical as this suggestion may be, it offers an explanation of the diverse results obtained from different experimental herds. Johansson (1931) for instance, found that at 3 weeks there was very little change in average weight per pig for litters of 10–19 pigs. Below 10, the average weight increased fairly rapidly. Such results might be expected if high fertility and heavy milk production went together, or if there was special feeding of sows with large litters, for in both these circumstances the amount of milk and the average weight per pig (but

presumably not the economy of gain) would be relatively high in the large litters.

In the New Zealand results previously mentioned there was no definite change in average weaning weight with litter size. The explanation for this would appear to be that various breeds were used in the compilation of the results; those breeds characterized by low milk production and low fertility would depress the weaning weight of the pigs in small litters, while those of high fertility and milk production would raise the weaning weight of the pigs in large litters. The same thing could happen within a breed consisting of strains differing in fertility and milk production. Another factor which would probably tend to level out differences between litters is creep feeding with skim milk. The remarkably high weaning weights recorded in New Zealand are probably associated with supplementary feeding, and, under these conditions, it may be expected that differences in the amount of milk supplied by the sow will not be reflected to the same extent in the weaning weights.

It is perhaps worth pointing out here that although the numbers of litters containing four or fewer pigs are usually so small that little reliance is placed on them, data from various sources agree in showing a lower growth rate for very small litters than might be expected (*Bull. N.Z. Dep. sci. industr. Res.*, 1930; Blissett, 1932; McMeekan, 1936). The 3-week weights of Johansson (1931) and Wild (1927) also show a decrease in passing from 3 in a litter to 2. Wild, however, records his highest 8-week weights for litters of 2. Lush *et al.* (1934), in their examination of birth weights, find the maximum occurring in litters of 3-4 with a distinct decrease with litters of 2. As Lush remarks, the conditions responsible for very small litters seem to be inimical to the development of very heavy pigs, but whether the conditions effective during gestation are also effective up to weaning has yet to be discovered.

#### FERTILITY AND MILK PRODUCTION

Hammond (1926) has pointed out that sows which are very fertile have usually a good milk supply. This may mean simply that as litter size increases, the number of teats used and the total quantity of milk produced rise. In so far as increased economy of feeding by the young pigs or early supplementary feeding counteracts the effects of a reduced amount of milk per pig, weaning weight will not be affected by increased litter size, and the more fertile sows will have the appearance of being particularly heavy milkers. On the other hand, it is conceivable that the milk yield of

very fertile sows is higher than it should be on this argument. The frequency with which large litters approach much smaller ones in weaning weight per pig suggests that there is possibly relatively more milk available for the large litters than would be expected if the increase were due entirely to more teats being used. Both fertility and milk yield are closely connected with the functioning of the pituitary gland, and it may well be that large litters and heavy milking go together because of the possession of an active pituitary. As a rough test of this possibility, the records were examined and two groups of sows selected from them, namely, those which raised 8-9 pigs, after farrowing at least 5 more than this number, and those which raised 8-9 pigs after farrowing not more than 11. Excluding gilts' litters, there were only 11 and 7 litters respectively fitting this description. The former, which should have had the heavier weaners, actually had the lighter. More instructive results could be obtained by raising large and small litters on sows of known high fertility and sows of known low fertility and recording birth weights and 3-week weights.

#### VARIATION IN WEANING WEIGHT IN LITTERS OF THE SAME SIZE

The interpretation of the meaning of variations in weaning weight has much practical importance. As between herds, of course, all differences could be attributed to feeding, climate and related factors, and particularly to the herdsmen. But within the herd it is of importance to know why there is such a large variation in the average weaning weight for litters of the same size.

From the figures in Table II it can be quickly calculated that the percentage of the total variance made up of differences between average weights of litters of the same size is approximately 78, 71, 68 and 41 per cent for first, second, third and fourth-ninth litters respectively. There appears to be a reduction in variance with increasing age of the sow, but this may well be due to the effect of eliminating the poorer sows. If this were so, the explanation that this portion of the variance could be largely attributed to differences in milk supply (as distinct from differences in milking capacity) of the sow would receive some support. Comparison of the variance (mean square) within classes with the total variance shows that with the exception of the last pair the differences are negligible. That is to say, the variation in average weight is just as great for any one size of litter as it is for all litters together, regardless of size. It would seem, therefore, that in the Edinburgh herd litter size is a relatively unimportant, although real, source of variation, except perhaps where old tried sows are concerned.

## LITTER SAMPLING

Although the essence of the litter-testing schemes for the improvement of performance in pigs is the raising of samples of litters under standard conditions, there appears to be very little information as to the relation between such samples and the litters from which they came. As a rule the choice of sampling method depends on the nature of the variation in the population to be sampled and on the degree of accuracy which it is desired to attain. The generally adopted plan of using four pigs from a litter seems to have arisen from the conflict of economic and biological considerations. It may well have been assumed that the larger the sample the better the estimate of the whole, but obviously the costs of maintaining establishments for raising larger samples would be excessive and the scope of the schemes would be reduced. In these circumstances the feasibility of litter testing may be regarded as dependent on the fact that a fair measure of the litter may actually be obtained from a sample no larger than four. Useful results might still be obtained of course, even if the sample itself were not of much significance, by the incidental focusing of attention on performance and husbandry.

Variation in pigs is well known to be associated with differences in breed or strain, breeding and feeding methods, climate and so on, so that the optimum sampling method will probably not be the same under all circumstances. Nor is it difficult to envisage a situation in which economic factors may make a sample of four too large, and then the question arises as to whether a smaller sample is worth while. In addition to the size of the sample, the way in which it is taken is open to variation. From a litter of 10 pigs for instance, it is possible to obtain 210 different combinations of four pigs. In practice a request is usually made that a "representative" sample of four "average" pigs should be sent to the Testing Station.

The Scandinavian Testing Stations ask for 4 pigs, 2 male and 2 female, which are close to the average weight for the whole litter. Reports from the Danish Experimental Laboratories (Beck, 1933) show that it is not always practicable to send in the ideal sample. The sexes are often not evenly distributed, and the variation in weight of the individual pigs may be rather large so that one or two animals in the sample depart widely from the average. The Danes apparently do not place so much importance on the actual similarity of the average weights of litter and sample, for they ask that the sample pigs shall be even and large for their age. This is

largely due to the fact that they observed that mortality was higher among the lighter pigs than it was among the heavier. Also the tests were not deemed to have begun until the pigs had reached 20 kg. in weight.

The Swedish Testing Stations (Bengtsson, 1934) required the sample to have the same average weight as the litter, and considered that if the discrepancy between them was not more than 1 kg. the accuracy of the test would not suffer. It was suggested that for the purposes of calculating the average of the litter, the commonly occurring runts or exceptionally small pigs should be neglected. The tendency for breeders to select pigs heavier than the average was considered a mistake, since the results of the test, although sometimes improved thereby, would give a less reliable estimate of the litter and of the breeding value of the parents. It was also rightly pointed out that the heaviest weaners did not necessarily give the best performance under test, for the factors governing their growth up to weaning were not the same as those governing it subsequently.

In a theoretical discussion of the accuracy of litter testing Lush (1936) states that the correlation between the average of a sample of  $n$  pigs chosen at random, and the average of the litter with  $t$  pigs, approaches

$\sqrt{\frac{nr}{1+(n-1)r}}$  when  $t$  becomes very large,  $r$  being the correlation coefficient for litter mates for the characteristic measured. Having obtained values of  $r$  it is possible to calculate from this formula the effects of increasing the number in a sample on the accuracy of the test as a measure of the whole litter. According to this process there is a rapid increase in accuracy as the sample increases from 1 to 3 with further, although smaller, increases up to 5. Beyond this the increases are small, and constantly become smaller as  $n$  increases. All this applies to random samples. According to Lush, "If the test pigs are *selected* with intent to get a *representative* sample, the correlations should be higher where  $n$  is small, but would not rise at so rapid a rate with  $n$ . If the samples from some herds are intentionally selected to do better than is really typical of the litter, but are not so selected in other herds, the correlations should be lower than those pictured but would rise more rapidly with increasing  $n$ ..." In what follows, it will be seen that actual sampling experiments give results which are in accord with these forecasts.

Since records have been kept of the growth of all pigs in the herd under discussion, and since the system of feeding has been kept constant, it is possible to calculate an approximate rate of growth for each pig by dividing the weight at completion of growth to bacon weight by the

## 494 *Weaning Weight and Litter Sampling of Pigs*

number of days from birth required to reach this weight. From these individual records means have been calculated for various types of samples and for the litters from which they have been drawn.

The types of sample used are as follows:

- (a) four pigs nearest the average of the litter;
- (b) three pigs nearest the average of the litter;
- (c) two pigs nearest the average of the litter;
- (d) four heaviest pigs;
- (e) four pigs chosen at random.

The first four samples were easily obtained. The random sample was secured by withdrawing numbered marbles from a hat in groups of four; the observed occurrences of the various numbers in test drawings were sufficiently close to the equality expected. All sampling was done without respect to sex. Lush (1936) has shown that as far as rate of gain is concerned, sex differences are negligible. After selection of the samples the mean rate of live-weight increase was found for each and compared with the mean for the whole litter, including the sample. This process gave the results presented in Table III.

Table III. *Mean daily rates of live weight increase obtained by various methods of litter sampling (at weaning)*

Rate of daily live-weight increase in lb.							
Litter size	No. of litters	Whole litter	Median four	Median three	Median two	Heaviest four	Random four
12	8	0.878	0.900	0.898	0.910	0.927	0.869
11	8	0.957	0.963	0.951	0.969	1.003	0.923
10	11	0.994	0.998	1.003	0.989	1.027	1.011
9	24	0.978	0.981	0.982	0.972	1.011	0.953
8	19	0.980	0.983	0.992	1.002	1.012	0.983
Loss of information %			8	16	34	15	10
Correlation between average rates of live-weight increase for samples and whole litters $r=0.96$				0.92	0.82	0.93	0.95

To facilitate comparison of the results Table IV has been compiled from the previous one by subtracting the mean rate of live-weight increase per day of the sample from that of the litter and multiplying the difference by 200 which gives an estimate of the average difference in weight at the end of 200 days between a pig of the sample and a pig of the whole litter. It then appears that the choice of the heaviest pigs for the test would give a better result (from the point of view of getting high performance from test pigs) than any other type of choice. This is to be expected in view of the positive correlation between the weight of a pig at weaning and its weight about 150 days later. The fact that the devia-

tions are all positive and comparatively large shows that such a sample does not give an accurate estimate of the litter as a whole. The actual difference, 6-11 lb., is, however, small. The remaining types of samples do not appear to yield any noteworthy differences.

Table IV. *Table showing mean differences between sample and whole litter multiplied by 200 to give an estimate of the average difference in weight at 200 days between pigs of the sample and of the whole litter. Differences calculated from the preceding table*

Litter size	No. of litters	Estimated mean difference in lb. from whole litter				
		Four pigs nearest average	Three pigs nearest average	Two pigs nearest average	Heaviest four pigs	Random four pigs
12	8	+ 4.4	+ 4.0	+ 6.4	+ 9.8	- 1.8
11	8	+ 1.2	- 1.2	+ 2.4	+ 11.2	- 6.8
10	11	+ 0.8	+ 1.8	- 1.0	+ 6.6	+ 3.4
9	24	+ 0.6	+ 0.8	- 1.2	+ 6.6	- 5.0
8	19	+ 0.6	+ 2.4	+ 4.4	+ 6.4	+ 0.6

The fact that all the differences were positive for the sample of 4 pigs nearest the average is probably without significance. Considering now the results from the standpoint of the litter classes, there does not appear to be any consistent change in any of the samples, with the possible exception of the largest litters (12 pigs), the samples from which gave positive and comparatively large differences from the litter averages (excluding the random sample). The performance of the random samples from litters of 11 and 12 pigs suggests that the occasional inclusion of the smallest pigs has been responsible for the distinction which has arisen between these samples and the remainder which do not include the smallest pigs. In litters smaller than 11 the occurrence of exceptionally small pigs is not so frequent, and therefore does not affect the random sample to the same extent.

In order to obtain a more comprehensive expression for the relation between performance of litter and sample, the coefficient of correlation between them was calculated from the average live-weight increase of each per day. The values of  $r$  given in Table III represent the degree of correlation irrespective of litter size. Before the data were combined an analysis of variance was made to determine whether there were significant inter-litter class differences in the behaviour of the sample. This was done by calculating the variance of the observed deviations of sample from litter average about the mean deviation. With the exception of the random sample, all cases gave no significant inter-litter class difference and the results were accordingly combined in the estimation of the coeffi-



cient  $r$ . For the random sample, the value of  $z$  corresponding to inter-litter class variance lay between the values for the 1 and 5 per cent points as given by Fisher (1936). This result arose from consistent negative deviations in litter classes 11 and 9, for which no explanation can be offered. The estimates of  $r$  were then made from each litter class separately, but since they were as similar as could be expected with random sampling in such circumstances, the values have been combined by means of the  $z$  transformation. From these values of  $r$ , "loss of information" has been calculated as equivalent to  $1 - r^2$ , and expressed as a percentage.

It is interesting to compare these results with the answer to the question of what was gained by testing four litter mates instead of any other number provided by Lush (1936) from intra-litter correlations. Using his expression for the value of the coefficient of correlation ( $r$ ) between average of litter and sample,  $\sqrt{\left(\frac{n}{t} \cdot \frac{1 + (t-1)r_{00}}{1 + (n-1)r_{00}}\right)}$ , and substituting values 2, 3, 4, for  $n$ , the number in the sample, and the value 0.3 for the coefficient of correlation ( $r_{00}$ , see later) among litter mates, we obtain 0.57, 0.69, 0.78 for the values of  $r$  for samples of 2, 3, 4 respectively, drawn from litters of 10 ( $t=10$ ). The observed values ( $t=8-12$ ) are much higher, namely, 0.82, 0.92, 0.96. These observed values are, however, derived from "representative" and not random samples, as stipulated by Lush. In this case, he expected that the correlations would "be higher where  $n$  is small, but would not rise at so rapid a rate with  $n$ ". This is partly borne out by his data, although the degree of correlation is rather higher than would be expected. The effect is to make samples of 4 sufficient for a very good estimate of the litter. To increase the sample to 5 would, on Lush's figures, give an appreciable improvement in the estimate, but in the present instance the improvement would certainly not be worth the trouble and expense. The high values of the coefficient require an explanation. The random sample which was taken yielded a composite value of 0.95 from components varying between 0.89 and 0.98, as compared with 0.78 expected from Lush's formula. The discrepancy apparently arises from the use of a coefficient of correlation between litter mates which is too small. If the coefficient were to rise to 0.5 then the corresponding calculated value of the coefficient of correlation between sample and litter would increase to almost 0.9 with a sample of 4. The intra-litter correlation coefficient has been worked out for the two largest litter classes and found to be 0.5 for litters of 8 and 0.3 for litters of 9. These facts, together with the size of the correlation between samples and litters, must indicate that within litters the pigs were of a high degree of

similarity. At this point opportunity might be taken to point out that to use 4 pigs for a sample will not give equally accurate results (*a*) for all herds, (*b*) for all litters, or (*c*) for all qualities of the pigs, since the intra-litter correlation will vary continually. If, as is sometimes supposed, there is an increase in variability as litter size increases, then a sample of 4 pigs should give a more accurate estimate for small litters than for large ones. There is some slight evidence of such an effect in the present data (Table IV).

#### RELATION BETWEEN WEANING WEIGHT AND SUBSEQUENT RATE OF GAIN

The foregoing comparison of samples and litters shows only that they behave in a very similar way, and does not concern itself primarily with the performances of the individual pigs. The intra-class correlation found for pigs within a litter has been seen to be for one litter class 0.3, and for another 0.5, indicating that within a litter there is a fairly strong tendency for the pigs to behave similarly. A somewhat different method of approaching the question is by estimating the regression of rate of live-weight increase on weaning weight. If there were a high degree of correlation between weights of pigs at weaning and at 200 days, the pig of average weight at weaning would still be the pig of average weight at 200 days of age. Litter testing would then require only one or two pigs from each litter. In practice, however, more are required. As the period of growth proceeds, therefore, there must be many changes in the order by weight into which the pigs sort themselves at weaning. As a measure of this, the regression on weight at weaning of rate of growth from weaning to 200 days was estimated for a portion of the population. Complete litters were used which were restricted in size to 8–10 pigs to avoid complications due to crowding and competition, and which gave a total of 203 animals treated in the calculations without further reference to litter. The regression of rate of live-weight increase on weaning weight was equivalent to 0.07 lb. per day for a difference of 1 lb. in weaning weight.

Table V. *Analysis of variance in rate of growth of sample of 203 pigs classified according to weaning weight*

Variance	D.F.	Sum of squares	Mean square	S.D.	$\log_e$	$z$
Linear regression	1	45.64	45.64	6.756	1.9104	1.6777
Deviations from regression	8	24.98	3.12	1.767	0.5693	0.3366
Within classes of same weaning weight	193	307.38	1.59	1.262	0.2327	—
Total	202	378.00	1.87	—	—	—

## 498      *Weaning Weight and Litter Sampling of Pigs*

An analysis of the variance is given in Table V, which shows that the variance of pigs of the same weaning weight was 1.59 (s.d. = 1.26).

The probability that a value of  $z$  as large as 1.67 would occur by chance is much less than 0.1 per cent. A linear regression, however, appears insufficient to express all the facts, for, on estimating the non-linear regression variance, a value is obtained which is large enough to reach the 5 per cent point of significance.

The intra-class correlation obtained from  $\frac{1.87 - 1.59}{1.87}$  is 0.15. This is

one way of expressing the fact that there was a considerable range of variation in the subsequent rate of growth of pigs of the same weaning weight. In other words, many pigs which are heavy at weaning do not maintain their relative superiority, and herein lies the difficulty of selecting pigs for breeding at early stages. Several factors affecting weaning weight are dealt with in the first part of this paper, and there are without doubt many others. Consequently, it is not easy to decide why a pig is small at weaning, but the factor or factors responsible must be supposed to have some bearing on its future performance. Causes of poor pre-weaning growth that may have lasting effects, preventing an animal from doing well under any circumstances, may be genetically inferior stamina or permanent injury following starvation or disease. Other causes may not be accompanied by inability to respond to improved environment. Included here would be various nutritional deficiencies of quality or quantity. Pigs affected by the latter may well show enhanced growth after weaning. That such cases are common is apparent when weight records taken at frequent intervals are available. These often show that for some reason or other an animal receives a check to growth and then recovers. Frequent occurrence of this phenomenon would explain much of the high intra-class variance shown in Table V.

### ANALYSIS OF VARIANCE IN GROWTH RATE FROM BIRTH TO 200 DAYS

An analysis of variance has also been made of the growth of pigs belonging to litters of 8 and 9, the growth rate in this case being calculated from the weight at completion (about 200 lb.) and number of days (about 200). In this way an estimate of the variance between litters and of the correlation between litter mates has been obtained.

For both litter classes the values of  $z$  are significant, the probability of such values being reached by chance being much less than 1 in 1000. There is, therefore, a part of the variance which is not accounted for by

random sampling of litters with a variance of their own. This may be expressed in another way by saying that there is a significant correlation between litter mates.

Table VI. *Analysis of variance in rate of growth from birth to bacon weight*

Variance	D.F.	Sum of squares	Mean square	S.D.	log S.D.	z
Litters of 8 pigs						
Between litters	18	1.5546	0.0864	0.2939	2.7753	1.1292
Within litters	133	1.2008	0.0090	0.0950	3.6461	—
Total	151	2.7554	0.0183	0.1351	—	—
Litters of 9 pigs						
Between litters	23	1.3088	0.0569	0.239	2.5686	0.7759
Within litters	192	2.3148	0.0121	0.110	3.7929	—
Total	215	3.6236	0.0168	0.130	—	—

The information may be summarized as follows:

	Litters of 8	Litters of 9
(1) Mean daily gain	0.987 lb.	0.986 lb.
(2) Standard deviation:		
(a) All pigs	0.135	0.130
(b) Pigs in a litter	0.095	0.110
(3) % of total variance due to differences between litters	56 %	36 %
(4) Correlation between litter mates	0.52	0.29

It will be noticed that except for the differences between litters and the correlation which depends on it, there is considerable similarity in the performance of the pigs of the two classes. The differences between litters amounting to 56 and 36 per cent are not significantly different from each other. The total variance found here for the growth rate from birth to 200 days is practically the same as that for the sample of 200 pigs mentioned in the previous section, whose growth rate was calculated from weaning to 200 days.

## DISCUSSION

It is pertinent to ask whether the intra-litter correlations discussed have any significance from the point of view of breeding, and, if so, to what extent they are an indication of the possibilities of improvement by selection. The existence of an intra-class correlation here indicates that the portion of the total variance existing between litters is not wholly accounted for by random sampling. There are, therefore, differences between litters, and a degree of similarity of litter mates which must be

attributed to certain factors which affect them, but not other pigs. Such factors are genetic and environmental, the latter including the various aspects of the mothering ability of the sow. With the present data it is not possible to arrive at an estimate of the relative importance of these two types of factors, but there is a certain amount of evidence from other sources. Assuming that rate of growth is governed by many genes with additive effects, and that the effects of dominance, epistasis, and correlation between parents' genotypes are negligible, the correlation between litter mates,  $r_{00}$ , is equal to  $e^2 + h^2/2$ , where  $e$  represents environmental and  $h$  hereditary effects (Lush, 1931). If  $e^2$  were zero,  $h^2$  would reach a maximum of 0.6 when  $r_{00}$  was 0.3, as it was found to be for a particular case. This 0.6 would then represent the portion of the individual variance which was due to additive gene effects. This value is almost certainly too large because environmental effects on such a character as rate of gain are very probable and would influence it more than such a character as body length. From his investigations of the results of Danish Litter Testing Stations, Lush (1936) comes to the conclusion that about 25 per cent of the total variance may be attributed to genetic causes. Since the litter-mate correlation was estimated at 0.24, the portion of the variance due to  $e^2$  becomes 0.12, that is about 12 per cent. With somewhat larger values for  $r_{00}$ , such as those obtained, either  $h^2$  or  $e^2$ , or both, may be greater, but in any case there would seem to be room for improvement by selection. It might be expected that, applied to rate of gain, selection could operate in two ways, first, by changing the mean growth rate as a result of breeding from genetically superior animals, and secondly, by increasing milk yields of sows; for if rate of gain is calculated from birth to age at bacon weight,  $e^2$  will include variance due to differences in milking capacity of sows which itself is probably subject to additive gene effects.

The intra-litter correlations found by Lush (1936) and Berge (1936) are lower than those observed in the Edinburgh material. Owing to the comparatively small material, the sampling errors attached to the latter are rather high, so that the values of 0.3 and 0.5 obtained are not significantly different. Even the lower of these is larger than the values of 0.24 and 0.19 obtained by Lush and Berge respectively. Some of the differences may be accounted for by the fact that the rate of growth in the Edinburgh material was calculated from birth, whereas the Danish and Norwegian growth rates were calculated from beginning of test after weaning. The correlation derived from them does not, therefore, include differences of environment between litters up to weaning.

On the assumption that the Edinburgh herd is typical of the pigs of

Great Britain, it must be concluded that the success which has followed litter testing in Scandinavian countries would be repeated here. Differences in growth rate (which are probably partly genetic in origin) exist, and it may be assured that except for minor differences they can be detected with considerable accuracy by samples of 4 pigs per litter tested.

#### SUMMARY

1. Following an examination of weaning weight with respect to litter size, it is concluded that no general relation between the two exists. Although a significant regression of weight on litter size was found, it appears probable that in herds where the relation between fertility and milk yield is different, such a regression will not necessarily be found.

2. A sampling experiment was carried out to determine the extent to which a sample might be expected to represent the whole litter. A correlation of 0.96 was found between the mean growth rate of samples consisting of the four pigs nearest the average at weaning and the mean of the whole litter. This represents a loss of 8 per cent of the information. With three or two pigs chosen in the same way the loss was greater. Samples of four pigs chosen at random did not give results significantly different from those of the four pigs nearest the average. The choice of the heaviest four pigs resulted in a loss of 15 per cent of the information.

3. The slight difference between the results from the random sample and the sample of the four average pigs indicated that there must have been only a small correlation between weaning weight and subsequent rate of growth. An analysis of post-weaning rate of growth showed that the intra-class correlation of pigs of the same weaning weight was 0.15. Individual weight at weaning would therefore appear to be of slight value in estimating subsequent performance.

4. Intra-litter correlations of growth rate for litter classes eight and nine were found to be 0.3 and 0.5. These values are higher than those found by others, probably as a result of the inclusion of the pre-weaning period in the calculation of the growth rates. There would appear to be grounds for believing that at least a fifth of the individual variance may be accounted for by additive gene effects.

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# AN INVESTIGATION OF NITROGEN UPTAKE IN MIXED CROPS NOT RECEIVING NITROGENOUS MANURE

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(With Plate VII)

INVESTIGATIONS performed during the last ten years (mainly at Valios Laboratorium, Finland, and at Rothamsted Experimental Station) have established that benefits are in some cases derivable by a non-legume growing alongside an inoculated legume in sand culture. These benefits are expressed by a gain of nitrogen and better growth of the non-legume, compared with the same species of non-legume grown without nitrogen either in single culture or in the presence of an uninoculated leguminous species. The effect of an inoculated companion legume on a non-legume may thus be similar to that obtainable by the application of nitrogenous manure to a non-legume in single culture; it appears that the leguminous component of a mixed crop can sometimes confer part of its nitrogen on the non-legume. This effect has not been demonstrated in the field, but certain Rothamsted experiments<sup>(5)</sup> have shown that there was no significant gain in total nitrogen in a mixed crop when nitrogenous manures were applied.

Lipman<sup>(2)</sup> suggested as long ago as 1910 that legumes may vary in their ability to act as donors of nitrogen to companion non-legumes. The converse suggestion, that non-legumes may vary in their ability to utilize the nitrogen made available by companion leguminous plants, has been made by Nicol<sup>(3)</sup>. Nilsson-Leissner<sup>(4)</sup> has shown that there are both varietal and specific differences in the ability of some grasses to utilize inorganic nitrogenous manures, and that these differences themselves vary according as to whether the grass is grown in presence or absence of clovers in the field.

The author has had the idea that just as legumes may vary in the ability to act as "donors" of their symbiotically fixed nitrogen, so some non-legumes may be better "acceptors" than others of such symbiotically



fixed nitrogen as is not taken up by the leguminous plants. The following paper contains two sets of experimental results obtained in an attempt to examine the influence of four species of nodule-bearing leguminous plants upon English rye-grass and barley in sand culture. The first experiment was performed at Puławy in 1935, the second at Rothamsted in 1936.

### I. EXPERIMENT AT PUŁAWY IN 1935

The experiment was carried out in pots containing 30 kg. of drift sand, in an unheated greenhouse. The sand had been washed four times in tap water, and twice in distilled water, before potting. The pots were divided into five series, each containing five replicates. The following nutrient salts were added in one dose to each pot before sowing:

$K_2HPO_4$	...	...	...	4.4 g. per pot
$KH_2PO_4$	...	...	...	0.3 „
$MgSO_4 \cdot 7H_2O$	...	...	...	0.3 „
KCl	...	...	...	0.3 „
$FeCl_3$ anhydride	...	...	...	0.075 „

The pH of the sand during the vegetation season was about 6.5. The seeds of the leguminous plants were inoculated with an effective strain of the specific nodule bacteria. The pots were watered with distilled water to a constant weight (60 per cent of the full water capacity of sand).

The scheme of the experiment is given in Table I.

Table I

Series	Plants
A	20 plants of English rye-grass
B	10 plants of English rye-grass
BC	10 plants of serradella and 10 plants of rye-grass
BD	10 plants of red clover and 10 plants of rye-grass
BE	10 plants of peas and 10 plants of rye-grass

Sowing was done on 1 July, and after 13 weeks' growth all plants were harvested on 30 September. At that time, serradella and clover were still in flower with some pods formed, and the peas were nearly ripe. The pots were left unwatered for several days before harvest, till the sand became dry, the plants were then lifted out of the sand, gently shaken and washed free from sand; the bulk of the sand was passed through a sieve to remove fragments of root.

Length of roots, air-dry weight and nitrogen content of plants and nitrogen content of sand were ascertained (unfortunately the sand was

not analysed before use), the results being summarized in Tables II and III.

Table II. *Average air-dry weight of rye-grass, g. per pot*

Series	Total plant		Shoot		Root g.	Overall length of roots cm.
	g.	as % of B	g.	as % of B		
A. Rye-grass (20 plants)	$1.25 \pm 0.01$	—	$0.61 \pm 0.06$	—	0.64	20
B. Rye-grass (10 plants)	$1.02 \pm 0.12$	100	$0.54 \pm 0.06$	100	0.48	19
BC. Rye-grass (10 plants) + serradella	$1.92 \pm 0.27$	188	$1.08 \pm 0.15$	200	0.84	22
BD. Rye-grass (10 plants) + clover	$2.14 \pm 0.12$	220	$1.17 \pm 0.06$	217	0.97	29
BE. Rye-grass (10 plants) + peas	$3.29 \pm 0.35$	322	$1.72 \pm 0.16$	331	1.57	24

Table III. *Total and percentage nitrogen in dry matter of rye-grass per pot*

Series	Nitrogen %	Nitrogen mg.	Nitrogen as percentage of nitrogen in B
B. Rye-grass (10 plants)	0.78	7.96	100
BC. Rye-grass (10 plants) + serradella	0.92	17.66	222
BD. Rye-grass (10 plants) + clover	1.05	22.47	282
BE. Rye-grass (10 plants) + peas	1.17	38.49	483

It is evident that the leguminous plants grown with rye-grass had a significant effect upon the total yield and nitrogen yield in rye-grass. The best companion of rye-grass seems to be the pea (Pl. VII, fig. 1). In this series of pots (BE) the yield of rye-grass was three times, and the nitrogen yield about five times, as large as in the control pots. There was a little interpenetration of roots(1) in this series of pots. All roots were long and well developed.

In the clover series there was extensive interpenetration of roots, so that separation of the roots of the two species became very difficult. The effect of clover upon the rye-grass growth and nitrogen yield (as well as nitrogen content) was less marked than in the previously described series of pots.

In the serradella group the roots of rye-grass were badly developed and short, the tops were light green, but even so the yields of grass and of total nitrogen in the grass were twice as great as in the pots with rye-grass only.

The sand of the pea-rye-grass pots contained the most nitrogen after harvest (Table IV).

Table V represents the yield of leguminous plants per pot, and percentages and yield of total nitrogen.

Table IV. *Nitrogen in dry sand after harvest*

Series	%	g. per pot (29 kg. dry sand)
B. Rye-grass	0	0
BC. Rye-grass + serradella	0.0015	0.44
BD. Rye-grass + clover	0.0011	0.32
BE. Rye-grass + peas	0.0042	1.22

Table V. *Leguminous plants: yield and nitrogen content as percentage of dry matter*

Plants	Dry matter g. per pot	% nitrogen	Nitrogen g. per pot
Serradella	8.25	3.07	0.24
Red clover	23.70	3.20	0.75
Peas	77.31	2.66	1.97

Summarizing the nitrogen yield in (1) leguminous plants, (2) rye-grass, and (3) sand (per pot), it is evident that ten plants of peas have given the highest amount of assimilated nitrogen and ten serradella plants the smallest amount. On the other hand, per 100 g. of plants, the peas and red clover fixed a smaller total amount of nitrogen, as is shown in Table VI.

Table VI. *Total symbiotically fixed nitrogen during 13 weeks' growth*

Series	Nitrogen fixed by 10 plants	Nitrogen per 100 g. of legu- minous plant (dry matter)
	mg.	mg.
Serradella	702	8500
Red clover	1102	4640
Peas	3218	4160

## II. EXPERIMENT AT ROTHAMSTED IN 1936

The experiment was performed in glazed earthenware pots, each holding about 20 kg. of sand, containing 0.0026 per cent of total nitrogen. 10 g. of precipitated chalk was well mixed with the sand before filling each pot, and 500 c.c. of the following nutrient solution was also added:

$K_2SO_4$	...	...	1.75	g.
$K_2HPO_4$	...	...	0.75	„
$KH_2PO_4$	...	...	0.75	„
$FeCl_3$ anhydride	...	...	0.075	„
$MgSO_4 \cdot 7H_2O$	...	...	0.75	„
$CaSO_4 \cdot 2H_2O$	...	..	0.6	„
$NaCl$	...	...	0.75	„

The pots were weighed at the commencement of the experiment, and afterwards watered with rain water as required to bring them back to the initial weight. The experiment was divided into five series, each containing five replicates, as shown in Table VII.

Table VII

Series	Plants
A	10 plants of barley
B	5 plants of barley
BC	5 plants of barley and 5 plants of peas
BD	5 plants of barley and 5 plants of lucerne
BE	5 plants of barley and 5 plants of red clover

The *pH* of the sand during growth was about 6.5. The seeds were inoculated with an effective strain of specific nodule bacteria. The plants were grown as described in the first experiment, with sowing 17 March and harvest 4 July. The stages of growth of the harvested plants were as follows: the barley was quite ripe in all pots, peas ripe and partly dry, red clover and lucerne flourishing and still green. The approximate dates of plant development are given in Table VIII.

Table VIII

Plants	Germination	Flowering	Maturity
Barley	24 March	—	3 July
Peas	29 March	15 April	15 June
Lucerne	24 March	3 July	—
Clover	23 March	3 July	—

The experimental results are given in Table IX, and the nitrogen determinations in Table X.

Table IX. *Mean weight of barley (dry matter) per pot*

Series	Total plant		Grain		Root g.	Overall length of roots cm.
	g.	as % of B	g.	as % of B		
A. Barley (10 plants)	3.00 ± 0.33	—	0.55 ± 0.10	—	0.32	32.90
B. Barley (5 plants)	2.87 ± 0.19	100	0.70 ± 0.10	100	0.27	40.30
BC. Barley (5 plants) + peas	4.28 ± 0.41	149	1.40 ± 0.18	200	0.50	49.40
BD. Barley (5 plants) + lucerne	3.18 ± 0.27	106	0.77 ± 0.13	110	0.40	39.03
BE. Barley (5 plants) + clover	2.80 ± 0.16	93	0.70 ± 0.14	100	0.40	39.05

Only in the peas-barley series was there a beneficial effect from mixed cropping, it being shown in that series as an increased yield of barley grain (Pl. VII, fig. 2). Though the roots of barley (in barley-pea pots) were well developed, no interpenetration of root system was observed. In the

other series (lucerne and clover pots) the leguminous plants had no evident effect on the growth of the barley.

Once more, regarding the nitrogen percentage and content of the barley, only in the pots with peas was there a demonstrable effect of the leguminous companion (Table X).

Table X. *Nitrogen percentages and total nitrogen in barley (dry matter)\**

	Series	Nitrogen %	Nitrogen mg. per pot	Nitrogen as % B
A.	Barley (10 plants)	0.55	16.50	—
B.	Barley (5 plants)	0.51	14.64	100
BC.	Barley (5 plants) + peas	0.87	37.24	254
BD.	Barley (5 plants) + lucerne	0.52	16.54	113
BE.	Barley (5 plants) + clover	0.59	16.52	113

\* These analyses were made on bulked samples.

### III. DISCUSSION

In both experiments peas have been the best companion of rye-grass and barley in their respective years. This beneficial influence of peas, as well as the less well marked effects of red clover and serradella, upon rye-grass, is explainable on the supposition that rye-grass, being a perennial plant, was able to utilize the nitrogenous compounds excreted from nodules of its leguminous companion during its whole season of vegetation.

Tables III and VIII show that in the conditions of these experiments rye-grass was better fitted than barley to make use of the nitrogen provided by a companion crop of peas. The yield of total nitrogen in rye-grass per pot in the peas-rye-grass series was nearly five times as big as the total nitrogen contained in grass grown alone; in the barley-peas series the nitrogen in the barley was only about 2.5 times as great as that of the barley grown in absence of peas.

In a study of root habits of mixed species, Kaserer<sup>(1)</sup> observed a maximum of root interpenetration when a legume and non-legume were grown together. Thornton and Nicol (see (6)) have made similar, though less extensive, observations. In the experiments described in the present paper, considerable root interpenetration was seen only in the rye-grass-clover pots. Little or no interpenetration of roots was noted in the other series; in spite of that, the associated growth of peas with rye-grass had a better influence upon the development of grass than did the association of clover with rye-grass.

The failure of lucerne and clover to influence the yield and nitrogen

content of barley grown in mixture with them under the conditions of this experiment may perhaps be accounted for as follows: it is known that the blooming period of leguminous plants is also the period of most vigorous fixation of nitrogen by the nodule bacteria; probably the excretion of nitrogenous compounds from nodules is most active at the flowering stage. By the time that in the foregoing experiment red clover and lucerne were in the blooming period, the accompanying barley had already ripened and was unable to utilize the available nitrogenous material. In other words, an excretion of nitrogen compounds from nodule-bearing plants (lucerne and clover) began at a stage of growth of barley that was much too late for the barley to benefit therefrom. The beneficial influence exerted upon barley by peas was evidently due to the circumstance that the period of flowering of peas, and the most vigorous growth of barley, almost coincided.

A non-legume cannot function as indicator plant towards nitrogen if it has reached a stage of growth at which its uptake of nitrogen is small.

In carrying out experiments with mixed crops it is therefore essential to associate species of plants (one leguminous, the second non-leguminous) having approximately parallel and equal periods of vegetation. As an alternative, should the duration of growth of the two companion species differ, the sowing dates should be modified, the plant having the longer period of growth being sown first by a suitably calculated interval. If neither of these precautions is adopted, a mixed crop experiment as a test of the presence of nitrogenous excretions from the leguminous component will have its value seriously reduced or even nullified.

#### IV. SUMMARY

1. The total yield of dry matter of rye-grass, grown in the presence of inoculated (*a*) peas, (*b*) red clover, (*c*) serradella, in sand with no added nitrogen, after 13 weeks' growth was increased by about three times in the peas-rye-grass series, twice in the clover-rye grass series, and nearly twice in the serradella series in comparison with the yield of rye-grass grown alone.

2. The nitrogen percentage and total nitrogen yield of rye-grass were greatly influenced by associated growth with peas, clover or serradella. Rye grass grown with peas after 13 weeks' growth contained nearly five times, grown with clover three times, grown with serradella about twice, as much total nitrogen, as grass of the same age similarly grown, but in the absence of leguminous plants.

3. Among the three leguminous species tested at Puławy, peas were the best companions for rye-grass, giving the highest amount of assimilated nitrogen, while serradella gave the smallest.

4. In another experiment, when barley and (a) peas, (b) red clover, (c) lucerne were grown together in sand without added nitrogen, only peas exerted a beneficial influence upon the yield of dry matter and the nitrogen percentage and total nitrogen yield of barley.

5. No influence upon barley growth was noted in the red clover-barley and in the lucerne-barley series. This was probably due to the circumstance that the period of most vigorous fixation of nitrogen by clover and lucerne nodule bacteria almost coincided with the period of ripening of barley, and at this stage of growth barley was unable to utilize the available nitrogenous compounds.

6. Rye-grass made a better use than did barley of nitrogen provided by peas grown in association.

7. An extensive root interpenetration in the clover-rye grass pots was noted. There was little or no root interpenetration in the other series of experiments with barley.

8. Certain precautions in the conduct of mixed cropping experiments are adumbrated.

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Fig. 1. Rye-grass grown in sand, alone or in association with leguminous plants. No nitrogen given as manure. 0—alone; 1, with serradella; 2, with clover; 3, with peas.



Fig. 2. Plants grown in sand without nitrogenous manure. Pot 16, ten barley plants; pot 21, five barley plants; pot 19, five barley plants + five peas.





# A STUDY IN SOIL CULTIVATION. THE EFFECTS OF VARYING SOIL CONSOLIDATION ON GROWTH AND DEVELOPMENT OF RAIN-GROWN COTTON

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(With Four Text-figures)

## CONTENTS

	PAGE
Introduction . . . . .	511
Description of experiments and seasons . . . . .	513
I. Soil conditions :	
Soil density and consolidation . . . . .	516
Soil moisture . . . . .	520
Soil nitrates . . . . .	523
II. Plant growth . . . . .	524
Germination . . . . .	525
Plant development. Sampling data . . . . .	525
(a) Growth and development. Primary data . . . . .	526
(b) Growth and development up to first flowering. Derived data . . . . .	529
(c) Lateral roots . . . . .	533
Final yield . . . . .	534
Statistical analysis . . . . .	535
General discussion . . . . .	536
Summary. . . . .	539
Acknowledgements . . . . .	540
References . . . . .	540

## INTRODUCTION

THE importance of the physical texture of the soil in determining plant growth is a question of primary interest to the agriculturist. Progress of research has to a large extent laid bare the principles and practice of the control of yield by the application of artificial manures, so that the effect of cultivation is once again at the focus of enquiry. The perfection of new implements in recent years has made a study of cultivation as apart from fertilizer practice a matter of the greatest concern. It must be admitted that little precise knowledge on this point is available even in regions

where agriculture is of old standing, and therefore the almost complete absence of information under tropical and subtropical conditions is not surprising. It is a truism to state that systems of agriculture must be adjusted to the requirements of local conditions, and for this reason every principle of agricultural science well enough established for temperate conditions must be scrutinized with the greatest care before its application on a large scale in the tropics can be advocated. In this connexion the question of artificially disturbing the soil by cultivation is probably the most important. The importance of aeration of the soil so much stressed by Howard (18) in India still requires confirmation elsewhere by precise experimentation. The experiments described in this paper are directed to an elucidation of the problem of cultivation practice for cotton under subtropical conditions. The experiments have been designed to take advantage of recent progress in experimental technique developed by Fisher (11) and in the analysis of growth processes by the methods of crop physiology instituted by Balls (1,2).

A rapid survey of investigations dealing with problems cognate to those here considered follows.

Comparatively little experimental work has been carried out to investigate the effects of different physical soil conditions, as obtained before sowing, upon the growth and development of the plant. Eden & Maskell (9) investigated the effects of natural variations in soil resistance, measured by the plough dynamometer, upon the establishment and development of the succeeding crops. During establishment of winter-sown wheat a negative correlation of plant performance with ploughing draught was found, but this effect diminished during growth and by harvest had vanished owing to the compensating effect of the spacing factor. With swedes no effect was noted. Keen (20) found that rotary cultivation improved germination and early growth of swedes and barley but had, if anything, a lowering effect on yield. Davies (7) obtained lower yields of oats after the same treatment. Culpin (5), measuring soil resistance by draw-bar pull or by means of a probe, found a negative correlation with germination of oats. Gupta (15), in pot cultures of beans and oats, found no effect of soil density on growth of tops but a modification of the root system. The author is unaware of any published work on the subject so far as cotton is concerned, or indeed for any tropical or subtropical crop plant.

The present series of experiments was undertaken to investigate the effects upon the cotton plant of soil treatments designed to give different degrees of soil density and consolidation. It is therefore necessary in con-

sidering the data here presented to establish: (1) a real difference in the soil conditions between the experimental plots as a result of the treatments given, and (2) a difference in growth of the plants. To investigate the first aspect the physical characteristics of soil consolidation, depth of ploughed soil, moisture content, and in some cases nitrate content, were estimated. As to the second aspect, for agricultural purposes the establishment of yield differences between the treatments would have sufficed. At Barberton, however, owing to the vagaries of climate and insect attack, yield is not determined mainly by soil conditions, and any differential effects of treatment on yield are liable to be obscured. Evidence of treatment effect must therefore be sought mainly in the growth behaviour of the plants in their early stages. The work of Crowther<sup>(4)</sup> in the Sudan, where somewhat similar difficulties arise, has shown that from studies of vegetative growth (maximum leaf weight) and flower production, yield can be forecasted with considerable accuracy. The data of plant development not being of primary interest here will not be presented in full, and will be used only to establish real treatment differences in growth and to indicate the soil factors concerned.

The experiments were carried out in four seasons from 1932 to 1936. A dust storm and hail early in the season rendered the plant samples of 1932-3 of little value, while bollworm attack in season 1933-4 and probably the aphid infestation in 1934-5 and 1935-6 caused the plants to become very irregular in the later parts of those seasons.

The interpretation of the data is based on statistical estimations of the errors concerned. Incidentally, the statistical data thus obtained may be used to evaluate the efficiency of different measures of growth and also the loss of information entailed by the use of sampling methods in estimating the mean value of any characteristic of the whole plot. A detailed consideration of these problems will have to be deferred for the present. It is hoped to deal with them in a later paper.

#### DESCRIPTION OF EXPERIMENTS AND SEASONS

The experiments were carried out with rain-grown cotton at the Cotton Experiment Station, Barberton, during the four seasons 1932-3, 1933-4, 1934-5 and 1935-6. The soil is a medium red loam having a "field capacity" for moisture of about 20 per cent of the oven-dry weight.

In each season the experiment occupied a new portion of ground (about 2½ acres, except in 1935-6), and a dressing of 200 lb. per acre of superphosphate was applied. Ploughing (20-25 cm. deep) and all subse-

quent cultivations were carried out using a "Caterpillar Fifteen" tractor. A randomized block lay-out was used for the three treatments, the number of replications and size of plots being as shown below:

	1932-3	1933-4	1934-5	1935-6
Number of blocks ... ..	4	5	5	6
Size of plot, in feet (excluding guard rows and end plants) and number of plants per plot	188 × 24½	172 × 24½	172 × 24½	132 × 7
	658	602	602	132
Size of end portions sampled, in feet (excluding guard rows and end plants) and number of plants	62 × 24½	64 × 24½	64 × 24½	Whole plot sampled
	+	+	+	—
	62 × 24½	64 × 24½	64 × 24½	
	217 + 217	224 + 224	224 + 224	132

The plots stretched right across the field to facilitate cultivation.

The three treatments may be summarized as follows:

*Normal (N).* This represented the usual practice at the Cotton Experiment Station for the given moisture conditions, and consisted of harrowing after spring rains (with a peg-tooth or disk harrow according to the moisture), followed if necessary by a further harrowing just before planting. (In 1933-4 a cultivation with a "Sunshine" spring-tyne cultivator was also given to break up hard moist soil below about 8-10 cm. depth.) The Normal treatment in all seasons left the soil in a state of moderate consolidation.

*Grubbed (G).* After the Normal treatment a deep cultivation to ploughing depth was given with a large rigid-tyne cultivator of the grubber type (Ransome's). In 1932-3 a further cultivation with a "Sunshine" cultivator was given just before sowing. In all seasons the soil of the Grubbed plots was extremely loose and soft right down to ploughing depth at the time of sowing.

*Compressed (C).* In 1932-3 the Compressed treatment was not comparable with the *C* treatments in the other seasons and will be denoted by *c*. The compressing was carried out by a tandem disk-harrow (set practically straight) followed by a heavy peg-tooth harrow and a pulverizing roller. It left the soil in a much consolidated condition. In the three later seasons, the Normal treatment was followed by a disk-harrowing with the disks set straight or nearly so. The "Caterpillar" tractor was then run up and down the plots so that only about 8 cm. were left between any two track marks. A further disk harrowing was given to break up the surface sufficiently for planting. The *C* treatment left the soil very firm indeed, especially in the last two seasons when low gear was used. The consolidation of the soil at about 8 cm. depth appeared similar whether under or between track marks. In 1933-4 the soil

moisture at the time of compressing was 13.6 per cent at 8–13 cm. depth, and only 11.8 per cent at 15–20 cm. In 1934–5 the soil was much more moist: 17.0 per cent moisture at 8–13 cm. and 18.3 per cent at 15–20 cm. In 1935–6 the moisture conditions appeared very similar to those in 1934–5. No samples were taken.

The spacing in all seasons was  $2 \times 3\frac{1}{2}$  ft., accurate spacing being obtained by hand planting along marked wires. 920 S.B. cotton was used in the first three seasons and U4/4 New S.B. in 1935–6, both being selections out of U4, a type of Upland cotton that has been very successful at Barberton and elsewhere. The dates of sowing, germination, and thinning are shown below:

	1932–3	1933–4	1934–5	1935–6
Sowing	8. xi. 32	6. xi. 33	28. xi. 34	30. xii. 35
Germination	14. xi. 32	12. xi. 33	2. xii. 34	3. i. 36
(50 % or more)				
Thinning	31. xii. 32 (1 per hole)	12. xii. 33 (2 per hole) 27. xii. 33 (1 per hole)	14. i. 35 (1 per hole)	3. ii. 36 (1 per hole)

Details of rainfall and other climatic conditions can be obtained from the reports from Barberton for the appropriate seasons (8). The following notes will indicate the salient features of the four seasons:

1932–3. Very early conditions were good, but a dust storm on 4 December 1932 followed by heavy winds and hail on 15 and 16 December 1932 caused much defoliation and bruising, making the plants extremely irregular. A 5 weeks' drought occurred in January and February, but fair yields of seed cotton were obtained (mean 791 lb. per acre).

1933–4. Germination and early growth were excellent. A drought in February and early March was accompanied by an attack of American bollworm (*Heliothis obsoleta*) of almost unprecedented severity. The latter by early March had rendered the plants extremely irregular both as regards growth and fruiting. Owing to the recovery after the attack had passed off, the plots yielded an average of 783 lb. of seed cotton per acre, but with no significant effect of treatment.

1934–5. An excellent stand was again obtained, but aphid appeared early in the season, and although the plants on one half of the field continued to grow well those on the other half remained small and stunted. Much of this difference was eliminated by the arrangement of the experiment, but a considerable patchiness of growth also developed over the plots as the season progressed—again possibly due to irregularities in the aphid infestation. The mean yield was only 340 lb. of seed cotton per acre.

1935-6. The season opened very late. Germination was excellent, but aphid again appeared early in the season, followed by a great deal of irregularity and patchiness of growth. The yield was extremely small (86 lb. of seed cotton per acre).

## I. SOIL CONDITIONS

### *Soil density and consolidation*

(a) 1932-3, 1933-4 and 1934-5.

In order to obtain information as to the differences between treatments in the physical condition of the soil, measurements of soil resistance were carried out, at three positions per plot in 1932-3 and four positions in 1933-4 and 1934-5, using an apparatus described elsewhere (17). Briefly, the apparatus consisted of a cone-shaped probe which was driven into the soil by a weight falling through a constant distance. The number of impacts needed for unit penetration of the probe at any depth was previously taken as a measure of the soil consolidation<sup>1</sup> at that depth. With an apparatus of this type the resistance of the soil is not directly proportional to the number of impacts, there being an added constant due to the force exerted by the weight of the whole system. On the assumption that resistance is constant during the penetration due to one impact

$$R = iu^2 \frac{(w_1 + w_2)}{2} + (w_1 + w_2)g,$$

where  $R$  = resistance in dynes,  $u$  = velocity in cm./sec. at which the entire system starts to enter the ground as a result of the impact,  $w_1$  = mass of falling weight (765 g.),  $w_2$  = mass of probe entering ground (1497g.),  $i$  = number of impacts for 1 cm. penetration,  $g$  = 980 cm./sec./sec. For the apparatus used

$$R = i \times 23.17 + 2.22 \text{ megadynes.}$$

This equation, which is only an approximation in view of the above assumption, is in the form

$$R = i \times \text{a constant} + \text{a constant.}$$

Since such constants do not affect the *significance* of a difference between

<sup>1</sup> In two recent papers, Culpin (5, 6) uses the term compactness to mean the "density of the mass of soil", and consolidation to mean the complex characteristic which is measured by the resistance of the soil to penetration by implements or probes. In the paper cited (17) the term compactness was used in the wide sense that Culpin uses consolidation. It is proposed, therefore, to omit the term compactness altogether, and use density or consolidation in the senses that Culpin uses compactness or consolidation.

two treatments, the significances given in the previous paper for impacts per cm. are also correct for resistances calculated as above.

The results of the soil resistance determinations carried out in the first three seasons are shown graphically in Fig. 1. The method of interpretation of such curves has already been described in detail for the 1932-3 results (17) and need not be repeated here. The treatment means

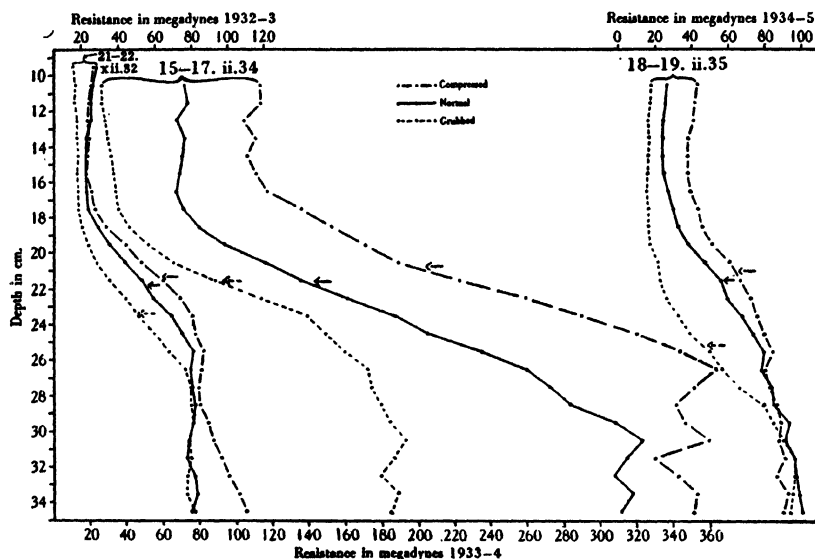


Fig. 1. Soil resistance determinations. Seasons 1932-3, 1933-4 and 1934-5.

over a range of depths such that the cone was entirely in the ploughed soil are shown in Table I, and for the soil below ploughing depth in Table II. The mean depths from the surface to the bottom of the ploughed soil, estimated from the individual resistance determinations as previously described (17), are given in Table III and indicated by the arrows in Fig. 1.

Table I. *Mean soil resistance in megadynes. Ploughed soil*

Season	Date	Depth cm.	Com- pressed	Normal	Grubbed	S.E.	Significant differences*		
			C	N	G		C - N	N - G	C - G
1932-3	21. xii. 32	9-17	24.8	24.7	17.5	0.58	.	++	++
1933-4	15. ii. 34	10-15	109.5	70.9	28.4	5.1	++	++	++
1934-5	18. ii. 35	10-17	39.8	25.2	18.7	1.93	++	+	++

\* Throughout this paper the following convention for indicating significant differences will be used: + or - for a positive or negative difference of significance  $P=0.05$  to  $0.01$ ; ++ or -- for differences of significance  $P<0.01$ .



Table II. *Mean soil resistance in megadynes. Below ploughing*

Season	Date	Depth cm.	Com- pressed	Normal	Grubbed	S.E.	Significant differences		
			C	N	G		C-N	N-G	C-G
1932-3	21. xii. 32	27-35	95.8	81.2	80.2	6.2	.	.	.
1933-4	15. ii. 34	29-35	347.4	314.0	186.7	36.8	.	+	+
1934-5	18. ii. 35	29-35	89.8	96.1	92.8	4.6	.	.	.

Table III. *Mean depth from surface to bottom of ploughed soil (cm.)*

Season	Date	Depth cm.	Com- pressed	Normal	Grubbed	S.E.	Significant differences		
			C	N	G		C-N	N-G	C-G
1932-3	21. xii. 32	—	21.3	21.8	23.3	0.35	.	—	—
1933-4	15. ii. 34	—	20.8	21.6	21.5	0.63	.	.	.
1934-5	18. ii. 35	—	21.0	21.5	25.2	0.61	.	—	—
1935-6	21. ii. 36	—	22.5	26.6	28.4	0.31	—	—	—

*Discussion.* In 1932-3, the determinations were made early in the season but after very heavy rains and hail (1.59 in. of rain and hail fell in under an hour on 15 December 1932). In 1933-4 and 1934-5 the measurements were carried out much later (after first flowering), when most of the rain for the season had fallen. The results therefore demonstrate the persistence of treatment effects in the ploughed soil after it had received a good deal of beating down.

The soil resistances for the different seasons are not directly comparable with one another owing to differences in the moisture conditions of the soil at the times of the determinations, and also because the cone used in the two later seasons was slightly different in shape from that used in 1932-3 (17). It should be noted, however, that the resistances found in the ploughed soil for both the Grubbed and Normal treatments were practically the same in 1934-5 as in 1932-3, whereas the Compressed treatment showed a much higher resistance in 1934-5. This was in all probability due to the much smaller consolidating effect of the method of compression used in 1932-3. There is no doubt that the very high resistances found for 1933-4 were due to the low soil moisture contents at the time.

It seems probable that *parts* of the treatment differences in resistance of ploughed soil were due to soil moisture. The treatment means for soil moisture determinations 18-23 cm. depth (i.e. at the bottom of the ploughing) are shown in Table IV.

It will be seen that the treatment differences are very small, except for 1933-4. In any case they cannot account for *all* the observed treatment effects on resistance of ploughed soil, since for 1933-4 Compressed

and Normal show a very significant difference of resistance with practically equal soil moisture, while for 1934-5 the same is true of Normal and Grubbed. It is concluded, therefore, that the soil-resistance measurements in ploughed soil show real treatment differences apart from soil moisture effects.

Table IV. *Soil moisture percentage of oven-dry weight. 18-23 cm. depth. (At or about time of resistance determinations)*

Season	Date	Com-pressed C	Normal N	Grubbed G	S.E.	Significant differences		
						C - N	N - G	C - G
1932-3	19. xii. 32	20.45	19.95	20.90	0.48	.	.	.
1933-4	15. ii. 34	10.84	10.62	12.64	0.35	.	- -	- -
1934-5	18. ii. 35	17.82	18.68	18.44	0.19	-	.	.

The significant treatment effects found for soil resistance below ploughing in 1933-4 were probably entirely due to soil moisture differences such as those found at 18-23 cm. (Table IV), or at 38-46 cm. on 8 December 1933 and 46-53 cm. on 8 January 1934. (See "Soil Moisture" section below.) The *N-G* difference at any rate cannot have been due to greater compression below ploughing depth by the tractor, for the Grubbed plots had one more run than the Normal.

Culpin(5) shows that soil density is only one of the factors concerned in consolidation as measured by soil resistance. He found that significant density<sup>1</sup> differences between gyrotilled and control plots persisted for 9 months, while soil resistance differences persisted much longer. Density differences are probably a major factor in the resistance differences found in our experiments, as the measurements were all made within 4 months from time of treatment. The treatment effects found for depth from surface to bottom of ploughed soil (Table III) were probably mainly due to differences in soil density, although it is likely that the extra depth on the Grubbed plots was *partly* caused by the grubber scratching up the bottom of the ploughing.

#### (b) 1935-6.

Culpin(5) estimated soil density by the penetrating power of bullets fired into the soil as measured by means of a stiff wire pushed down the hole. This method was tried in 1935-6 but was not found satisfactory for this soil, although the latter was reasonably moist at the time (16 per cent moisture). The bullets penetrated straight down until they encountered the plough sole when they were sharply deflected.

<sup>1</sup> Penetration tests with revolver. See (b) 1935-6.

It was found that when a stiff (8 gauge) wire held between thumb and finger was pushed into the soil, at a certain point a very marked increase of resistance occurred such that the thumb and finger slipped down the wire. It seemed almost certain that this represented the bottom of the ploughing, and measurements of the depth at which it occurred were carried out at twenty-two positions on each plot. The individual positions showed considerable variation as might be expected, but the plot means were very regular, and very significant differences were found between treatments. These values have been entered in Table III.

*Discussion.* The depths found for 1936 were all greater than those found for the corresponding treatments in the previous seasons (Table III). This effect was probably due to recent hoeing, which had raised the level of the soil between plants in the rows where the measurements were all made. In previous seasons the soil was flattened off to the general ground-level before the determinations, but in 1936 this was not done. Values from Table III establish the fact that the Compressed soil was more dense than the Normal. The Grubbed soil showed an even greater depth than the Normal, though this may in part have been due to the grubber scratching up the unploughed soil.

In all seasons, marked differences in general surface level as between treatments could be seen at the time of treatment and persisted well into the season.

### *Soil moisture*

#### *(a) Ploughed soil*

In 1932-3, samples for soil moisture determination were taken at 18-23 cm. depth (i.e. at the bottom of the ploughing) on the second or third day after each rain and then weekly until further rain occurred. During the first half of the season (22 November 1932 to 17 January 1933) a very significant average treatment was found (Table V) but no interaction with occasion. Later in the season (February-March) a marked

Table V. *Soil moisture percentage of oven-dry weight. 18-23 cm. depth. (Means over several occasions)*

Season	Dates	Number of occasions	Com- pressed	Normal		S.E.	Significant differences		
			C	N	G		C-N	N-G	C-G
1932-3	22. xi. 32 to 17. i. 33	9	17.54	17.54	18.25	0.13	.	--	--
1933-4	15. xi. 33 to 22. iii. 34	6	15.03	15.07	15.93	0.13	.	--	--

interaction between treatment and occasion appeared and was due to the Grubbed treatment having a higher moisture content immediately after rain but a lower content at the end of a drought. The more rapid drying of the Grubbed soil was associated with the greater late growth of the plants on this treatment. The average treatment effect was now insignificant.

On four occasions, samples at 18–23 cm. depth were taken on the second or third day after 1 in. or more of rain. The treatment means are given in Table VI.

Table VI. *Soil moisture percentage of oven-dry weight (field capacity). 18–23 cm. depth. 1932–3. (Means over four occasions: 19 and 28 December 1932; 23 January 1933; 10 March 1933)*

Compressed <i>c</i>	Normal <i>N</i>	Grubbed <i>G</i>	S.E.	Significant differences		
				<i>c</i> - <i>N</i>	<i>N</i> - <i>G</i>	<i>c</i> - <i>G</i>
20.83	20.60	21.65	0.199	.	- -	-

In 1933–4, samples were taken at 18–23 cm. depth on six occasions during the season. The treatment means are shown in Table V.

In 1934–5, samples for soil moisture were only taken on three occasions. The only significant results are those for 18 February 1935 which show Normal more moist than Compressed at 18–23 cm. depth (Table IV).

In 1935–6, samples were taken at 15–20 cm. on four occasions, two being shortly after heavy rains and the others after 3 and 5 weeks respectively of dry weather. The last-mentioned showed no significant treatment effect. The other results are shown in Table VII.

Table VII. *Soil moisture percentage of oven-dry weight. 15–20 cm. depth. 1935–6*

Date of samples	Date of previous rains > 1 in.	Compressed <i>C</i>	Normal <i>N</i>	Grubbed <i>G</i>	S.E.	Significant differences		
						<i>C</i> - <i>N</i>	<i>N</i> - <i>G</i>	<i>C</i> - <i>G</i>
3–5. ii. 36	30–31. i. 36	18.85	18.23	17.27	0.248	.	+	++
21–22. ii. 36	30–31. i. 36	15.83	16.12	16.33	0.110	.	.	- -
24. iii. 36	22. iii. 36	20.48	19.65	19.40	0.142	++	.	++

(b) *Undisturbed soil below ploughing.*

In all seasons, samples for soil moisture were taken below the ploughing, those in 1932–3 being taken at 30.5 cm. intervals from 23 to 30.5 cm. down to 144 to 151.5 cm. Only in 1933–4 were any significant effects of treatment found. These results are given in Table VIII.

Table VIII. *Soil moisture percentage of oven-dry weight.*  
*Below ploughing depth. 1933-4*

Date	Depth cm.	Com- pressed <i>C</i>	Normal <i>N</i>	Grubbed <i>G</i>	S.E.	Significant differences		
						<i>C-N</i>	<i>N-G</i>	<i>C-G</i>
8. xii. 33	38-46	16.98	16.66	17.50	0.167	.	- -	.
8. i. 34	46-53	17.12	17.64	18.46	0.161	-	- -	- -

*Discussion.* In attempting to interpret the soil moisture results, use has been made of the conception of "field capacity" (24). It is assumed that the soil moisture content as determined on the second or third day after saturation represents the "field capacity", and that once this has been reached further movement of moisture, except removal by plant roots, will be slow. As is seen above (Tables VI and VII), the field capacity for the loam soil at Barberton is about 20 or 21 per cent of the oven-dry weight.

(a) *Ploughed soil.*

It is obvious that it is only possible to obtain the effects due to differences of soil structure, unaffected by those due to differences in plant size, by taking samples almost immediately after rain. The results obtained for the ploughed soil (i.e. the soil directly affected by the treatments) just after rains of 1 in. or more were, however, somewhat conflicting in 1932-3 and 1935-6. The results for the former season showed a significantly *greater* field capacity at 18-23 cm. for Grubbed than for Compressed or Normal (Table VI), while in 1935-6 samples taken on two occasions just after rains showed Grubbed significantly drier than Compressed at 15-20 cm. (Table VII). The latter effect cannot have been due to the size of plants, since the Grubbed plants were smallest, and shows that in 1935-6 the grubbed soil had a *smaller* field capacity at 15-20 cm. than the compressed.

The significant results for occasions other than those just after rain agree for all seasons (including 1935-6) in showing the grubbed soil more moist than the compressed. This may reasonably be attributed to the more rapid removal of moisture by the larger *C* plants.

On the whole, the treatment differences found were small, and it must be remembered that even when the Grubbed treatment was found to have a higher moisture content per unit dry weight it might well have a lower one per unit volume by reason of its less dense soil. No data were obtained for moisture content per unit volume, the technical difficulties making it impossible to do a large enough number of determinations.

*(b) Undisturbed soil below ploughing.*

The only significant treatment effect found was in the season 1933-4, when the Grubbed treatment was found to be more moist than the others on two occasions, at 38-46 cm. and 46-53 cm. depth respectively (Table VIII). These results might be explained by the smaller size of the Grubbed plants, although on the first occasion (8 December 1933, only 26 days from germination) the absolute differences in plant size were very small, the mean height being about 15 cm. It seems probable that the effect was partly due to the rougher surface of the Grubbed plots holding up run-off, and partly to the grubbed soil holding more excess water (above its field capacity) during rain and subsequently passing it on to the lower levels,<sup>1</sup> which would also reduce run-off. There is thus some indication that more water passes down through the ploughed soil on the Grubbed treatment.

*Soil nitrates*

Marked treatment effects upon the nitrate contents of the soil were expected, owing to differences in soil aeration. Nitrate determinations carried out in three seasons, by the phenoldisulphonic acid method, failed to confirm this. A summary of the data is presented in Table IX. No significant effects of treatment were found in any season, although on 3-5 February 1936 the average treatment effect ( $Z=0.6667$ ) and the treatment effect at 18-23 cm. ( $Z=0.6769$ ) approached the  $P=0.05$  level ( $Z$  required 0.7058).

Table IX. *Nitric nitrogen mg. per kg. air-dry soil, and soil moisture percentage of oven-dry weight*

Season	Date	Depth cm.	Nitric nitrogen			Soil moisture		
			Com- pressed	Normal	Grubbed	Com- pressed	Normal	Grubbed
			<i>C</i>	<i>N</i>	<i>G</i>	<i>C</i>	<i>N</i>	<i>G</i>
1932-3	20. ii. 33	18-23	29.3	36.0	33.2	13.4	12.8	13.1
1934-5	9-10. i. 35	18-23	4.9	4.5	4.0	18.2	18.5	19.2
		56-61	22.1	21.8	18.3	20.1	20.2	20.2
1935-6	3-5. ii. 36	18-23	5.3	4.8	4.5	18.9*	18.2*	17.3*
		30.5-35.5	10.8	10.0	8.5	20.0†	20.0†	19.8†
1935-6	20-21. ii. 36	15-20	6.0	5.8	5.8	15.8	16.1	16.3

\* 15-20 cm. depth. † 28-33 cm. depth.

The almost significant treatment effects found on 3-5 Feb. 1936 suggest that Compressed gave a higher nitrate content than Grubbed, but even on this occasion the differences between the treatment means

<sup>1</sup> Culpin (6) found that water penetrated into gyrotilled plots much more rapidly than into control plots.

were fairly small (Table IX). The insignificant effect due to treatment found in all three seasons is in agreement with the hypothesis that all the treatments gave equal mean nitrate contents in terms of weight of soil. Even were this so, on a volume basis the Compressed treatment must have had more nitrate in the ploughed soil than Grubbed owing to the greater density, and the treatment differences on a weight basis which almost attained significance on one occasion led further support to this view.

## II. PLANT GROWTH

The primary consideration in this paper has been to relate directly growth of the plant to the soil conditions obtaining. The available data as to soil conditions are presented above and in this section measures of plant development are considered.

As stated earlier, owing to the irregularities due to climate and insect attack, yield data in most cases failed to show significance. Developmental data, on the other hand, showed consistent differences between treatments. Measures of plant growth have been selected which give information directly on the progress of the important physiological processes (Gregory (13), Crowther (4)). These include: (i) Germination. (ii) Total leaf numbers and nodes on main axis. These are direct measures of meristematic activity in relation to the development of the plant. (iii) Numbers of flower buds + flowers, green bolls, and open bolls. These measures enable the relation between potential and actual yield capacity of the plant to be determined. (iv) Node number of first sympodial branch. This gives a measure of the stage at which the reproductive phase is entered. (v) Dry and fresh weights of the whole plant and its several parts. These give measures of the integrated activity of the whole plant. (vi) Height of main axis. Although this measure in itself gives no important information, yet the ease with which it may be obtained and the fact that it is highly correlated with total dry weight makes it the most convenient measure for determining any treatment differences. Together with estimations of node number, total height of plant enables internode length to be determined, which gives a measure of adequacy of water supply apart from nitrogen supply (Crowther (4)).

The estimations enumerated above were performed on random samples taken at fortnightly intervals (except in 1935-6) throughout the season. From these primary data certain derivative data were obtained; namely net assimilation rate, efficiency index and relative leaf growth rate. These give directly a picture of the changes of important physiological processes with age and their interaction with treatment.

It is impossible to present in full the data obtained. Where possible the results have been presented graphically.

### *Germination*

Stand counts were carried out on at least two rows per plot in each season. The "percentage stand" figures obtained do not represent percentage germination since four seeds were planted per hole (three in 1932-3), but a comparison of the treatment means affords information which is related with the rapidity and completeness of germination. These means are presented in Table X.

Table X. *Percentage stand*

Season	Days from planting	Com-pressed <i>C</i>	Normal <i>N</i>	Grubbed <i>G</i>	S.E.	Significant differences		
						<i>C - N</i>	<i>N - G</i>	<i>C - G</i>
1932-3	8	97.4	98.5	96.1	0.49	.	+	.
1933-4	11	99.2	97.7	95.4	1.05	.	.	.
1934-5	3	38.3	32.9	19.8	2.81	.	+	++
	4	91.7	93.4	91.8	1.04	.	.	.
	6	97.4	97.6	97.8	0.33	.	.	.
1935-6	4	97.2	97.0	92.4	0.83	.	++	++
	7	99.9	99.5	97.6	0.47	.	+	++

It will be noted that on the Grubbed treatment the seedlings appeared somewhat more slowly, and that in 1932-3 and 1935-6 its total germination was slightly lower. These effects were possibly due to a tendency to plant more deeply, in spite of careful supervision, in the very loose grubbed soil.

*Date of germination.* The date when the stand reached 50 per cent or more was taken to be the date of germination. In seasons 1932-3 and 1933-4 this was estimated by eye as occurring 6 days from planting (p. 515). As can be seen from Table X, in 1934-5 and 1935-6 the date of germination was 4 days from planting.

### *Plant development. Sampling data*

The sampling methods used have been described in detail elsewhere<sup>(16)</sup>. The two end portions of each plot were used for sampling (see p. 514) and the middle portion was retained with its stand unimpaired for final yields. In 1935-6, plant sampling was only carried out on two occasions, the whole plot being sampled.

Two independent "sampling units" were taken from each plot on each occasion, except in 1934-5 and on the second occasion in 1935-6 when three were taken. The size of "sample" (i.e. the aggregate of sampling units from one plot) expressed as a percentage is given in the



section on "Statistical analysis" below. The numbers of plants sampled on any one occasion were 240 and 360 before thinning in 1933-4 and 1934-5 respectively and half those numbers after thinning.

Although on some occasions counts were made of lateral roots (see (c) below), it was not possible to obtain weights of root systems and the following sections except (c) refer to tops only. The plants were all cut at ground level.

*(a) Growth and development. Primary data.*

In 1932-3 the effects of the dust storm and hail early in the season, combined with the inadequate size of the sample (1.4 per cent), rendered the sampling data so irregular as to be almost useless. In 1935-6 plant samples were only taken on two occasions. This section therefore deals mainly with seasons 1933-4 and 1934-5, and especially with the periods before maximum leaf weight. (The later samples were too irregular to be of much value, owing to the bollworm attack in 1933-4 and probably to the aphid infestation in 1934-5.)

The effects of the treatments upon growth and development in 1934-5, as shown by the primary data from the plant samples, are given graphically in Figs. 2 and 3.

It appears from the graphs that up to about the time of maximum leaf weight the order of the treatments is similar on most of the occasions and for all the measures given, Compressed being above Grubbed with Normal usually intermediate. In 1933-4 the curves are similar, except that those for the Compressed and Normal treatments lie close together with the Grubbed treatment much lower, whereas in 1934-5 the Normal curve is generally nearer to the Grubbed. For both years, however, Grubbed is significantly lower than Compressed for at least part of the season in respect of every measure given in the figures, except in the case of open bolls (see below). Up to time of maximum leaf weight, Compressed is only found significantly lower than another treatment ( $N > C$  and  $G$ ) in the case of number of leaves at 22 days in 1933-4 and Grubbed is never found significantly higher than another treatment. In general it may be said that in both seasons the grubbing caused slower growth and development than the compressing, and that whereas in 1933-4 there was little difference between Normal and Compressed, in 1934-5 the latter was distinctly the better treatment.

The data for open bolls (number and dry weight of open bolls, dry weight of seed cotton) show the treatments in the same order but the results are not statistically significant.

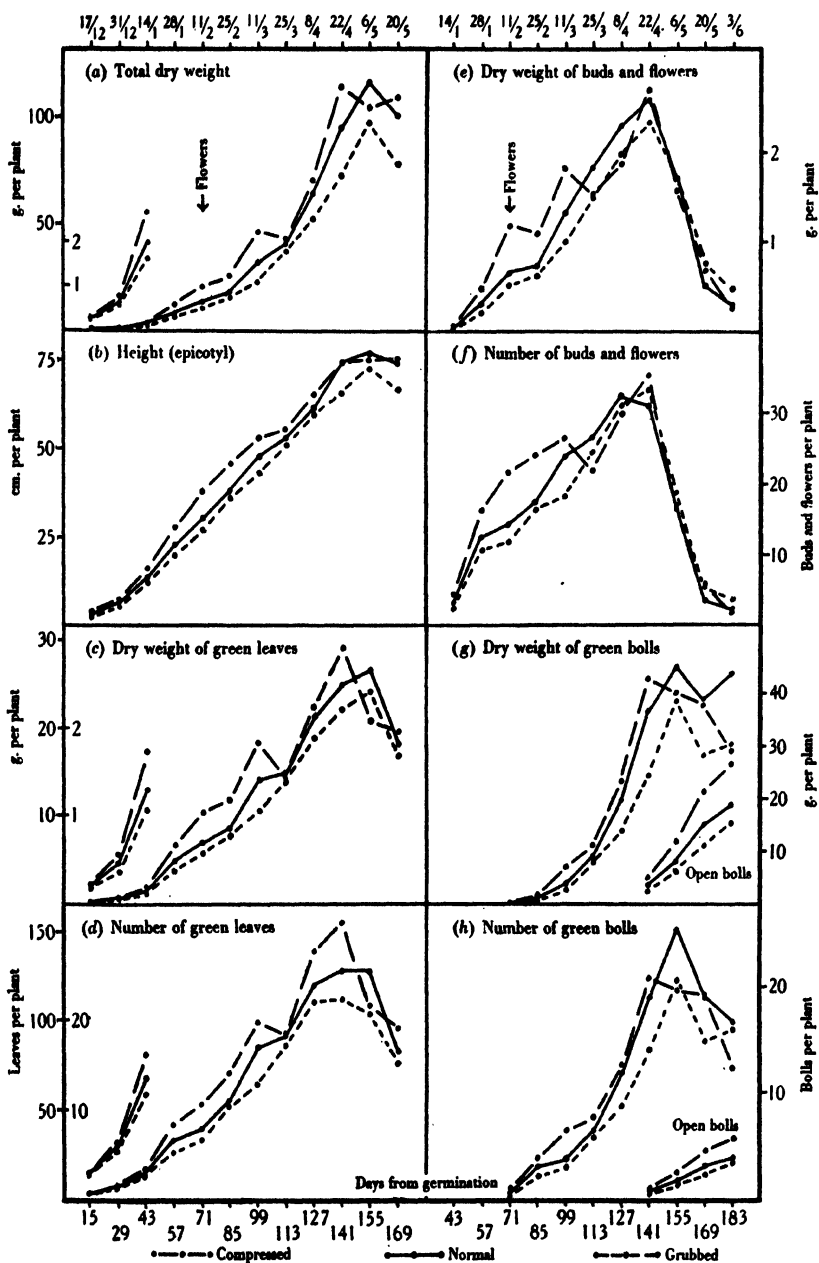
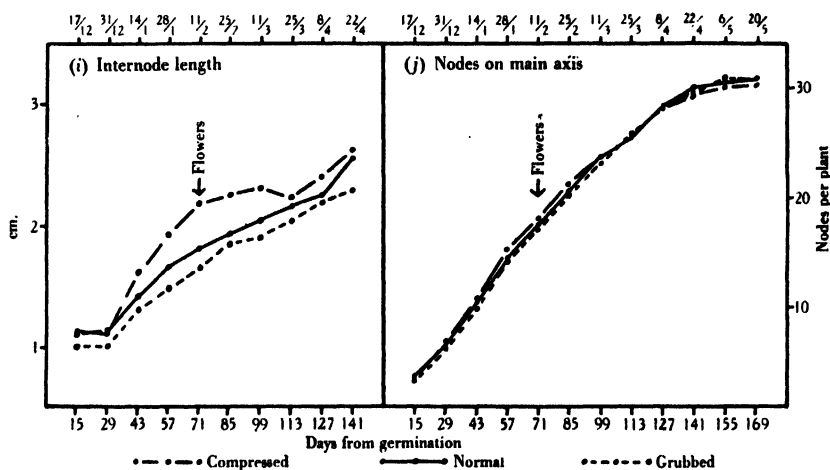


Fig. 2. Plant development, primary data. Season 1934-5.

In addition to the above-described quantitative treatment effects, a small but significant difference of habit was found in 1934-5 and 1935-6. The buds at the first few nodes of the main axis of the cotton plant, if



**Fig. 3. Plant development, primary data. Season 1934-5.**

they push at all, normally give rise to vegetative monopodia which repeat the structure of the main stem. Above a certain point, usually about the 6th node in the strains used, the buds normally give rise to sympodial fruiting branches. Thus a low node number for the first sympodial branch indicates an earlier fruiting habit. Counts of the node number of the first sympodial branch showed significant treatment effects, Compressed having a lower number than Grubbed. The treatment means are shown in Table XI.

Table XI. *Mean node number of first sympodial branch*

Season	Compressed <i>C</i>	Normal <i>N</i>	Grubbed <i>G</i>	S.E.	Significant differences		
					<i>C-N</i>	<i>N-G</i>	<i>C-G</i>
1934-5	6.88	7.22	7.58	0.130	.	.	--
1935-6	6.93	7.31	7.69	0.99	-	-	--

The other results yielded by the primary data for the two sampling occasions in season 1935-6 are indicated in Table XII. It will be seen that the results for the first sampling confirm those for the two previous

seasons in nearly every respect. For the second sampling the treatment means are in the order  $C > N > G$  for practically all the measures given, but the treatment effect only reaches a significant level for "dry weight of buds, flowers and bolls". This absence of significance was not due so much to lack of treatment effect as to the large variation associated with aphid attack.

Table XII. *Growth and development—primary data. Significances, etc.*  
Season 1935-6

Measure	Days*	Treatment means (per plant)				Significant differences		
		C	N	G	s.e.	C-N	N-G	C-G
(a) Total dry weight, g.	18	0.41	0.39	0.33	0.015	.	+	++
	66	7.26	6.70	5.42	0.64	.	.	.
(b') Hypocotyl length, cm.	18	7.2	6.6	6.3	0.12	++	.	++
	18	4.7	4.8	4.0	0.17	.	++	+
(b) Height main axis, cm. (Epicotyl)	66	19.4	18.8	16.8	1.0	.	.	.
	18	0.28	0.27	0.24	0.010	.	+	++
(c) Dry wt. leaves, g.	66	4.49	4.29	3.55	0.36	.	.	.
	18	3.57	3.61	3.25	0.089	.	+	+
(d) No. of leaves	66	23.4	22.8	20.6	1.5	.	.	.
	18	0.61	0.47	0.30	0.064	.	.	++
(e) Dry wt. buds, flowers and bolls, g.	66	9.33	8.22	7.15	0.72	.	.	.
	18	1.31	1.34	1.23	0.044	.	.	.
(f) No. of buds, flowers and bolls	66	1.48	1.43	1.31	0.054	.	.	.
	18	4.57	4.61	4.25	0.089	.	+	+
(g) Internode length, cm.	66	14.0	14.1	13.9	0.024	.	.	.
	18	14.0	14.1	13.9	0.024	.	.	.
(j) Nodes on main axis	66	14.0	14.1	13.9	0.024	.	.	.
	18	14.0	14.1	13.9	0.024	.	.	.

\* Approximate stages of development: third-fourth leaf, 18 days; first flower, 66 days.

(b) *Growth and development up to first flowering. Derived data.*

This section deals with the derived data from the first five of the fortnightly samplings in seasons 1933-4 and 1934-5, and with such additional information as was obtained from the two samplings in 1935-6. The following have been calculated.

(1) *Relative rate of total dry weight increase (efficiency index).* This is the linear regression coefficient of  $\log_e$  (total dry weight) on time, i.e. the average slope of the  $\log_e$  (weight) curve (Fig. 4). It is obvious from Fig. 4 that very nearly all the variation in time of  $\log_e$  (weight) can be expressed by a straight line. Statistical analysis carried out on the  $\log_e$  (weights) confirms that this is so, but shows that for mean  $\log_e$  (weight) over all treatments the deviation from linearity, although slight, is significant in each season. Since this curvature differs considerably in the two seasons,

however, it is probably due mainly to seasonal effects. In any case it is so small that it is reasonably accurate to consider the curves as straight lines and deal with their average slopes (linear regressions) only, i.e. to consider the total dry weight curves as exponential. Of the variation due to interaction of treatments and occasions (lack of parallelism between the treatment curves for  $\log_e$  (total dry weight)), the remaining portion due to deviation from linearity is quite insignificant for each season. For comparing treatments, therefore, the linear regression coefficients up to first flowering are entirely satisfactory measures.

(2) *Dry weight of green leaves percentage of total dry weight*: (3) *Dry weight per leaf*. As is the case for all the leaf data presented in this paper, only green leaves (and cotyledons where present) are included, since it was not possible to collect those shed.

(4), (5) *Water contents of green leaves and whole plants, percentage of dry weight*. In order to find the leaf fresh weights, the tins in which the plants were brought to the laboratory (18) had to be opened and the leaves cut off and weighed on a Joly balance. This must have led to considerable water loss and renders the leaf water contents somewhat open to doubt. As a check on these, the whole plant water contents have been investigated. The latter are quite reliable, the total fresh weights being obtained before the tins were opened.

(6) *Relative leaf growth rate*. This is calculated by a method analogous to that used for relative rate of total dry weight increase. As in the case of  $\log_e$  (total dry weight), the higher order interactions of treatment and occasion for  $\log_e$  (leaf weight) are quite insignificant, and it is therefore sufficient to use the linear regression coefficients for the comparison of treatments (Table XIII).

(7) *Net assimilation rate per unit leaf weight*. This is net assimilation rate calculated by Gregory's method (12) but on a basis of leaf weight instead of leaf area, i.e. increase in total dry weight per unit leaf dry weight per fortnight.

The treatment means up to first flowering for these derived data are presented in Table XIII.

In the following discussion all the effects mentioned are statistically significant, except where the contrary is expressly stated.

For each season the efficiency index falls slightly in time, as is shown by the quadratic regressions of  $\log_e$  (total dry weight) (Fig. 4), but no difference in rate of fall is found between treatments. The relative rate of total dry weight increase was higher for Compressed than Grubbed both in 1933-4 and 1934-5 (Table XIII and Fig. 4), while for 1935-6 the treat-

Table XIII. *Derived data up to first flowering. Treatment means*

Measure	Season	Com-pressed Normal Grubbed			S.E.	Significant differences		
		C	N	G		C-N	N-G	C-G
(1) Efficiency index (per 14 days)	1933-4	1.45	1.41	1.31	0.016	.	++	++
	1934-5	1.09	1.01	0.98	0.016	++	.	++
	1935-6	66.9	67.7	68.0	0.19	-	.	--
(2) Leaves % of total dry weight	1933-4	66.9	67.7	68.0	0.19	-	.	--
	1934-5	65.3	66.8	67.1	0.32	-	.	--
	1935-6	66.0	67.6	68.7	0.40	-	.	--
(3) Dry weight per leaf g.	1933-4	0.091	0.090	0.082	0.0015	.	++	++
	1934-5	0.109	0.100	0.094	0.0009	++	++	++
	1935-6	0.120	0.117	0.108	0.0029	.	+	+
(4) Water content % of leaf dry weight	1933-4	420.0	416.0	406.0	2.1	.	+	++
	1934-5	491.0	486.0	467.0	3.0	.	++	++
	1935-6	491.0	486.0	467.0	3.0	.	++	++
(6) Relative leaf growth rate (per 14 days)	1933-4	1.37	1.33	1.24	0.016	.	++	++
	1934-5	1.01	0.93	0.91	0.013	++	.	++
	1935-6	1.01	0.93	0.91	0.013	++	.	++
(7) Net assimilation rate (per 14 days)	1933-4	2.18	2.13	1.94	0.051	.	+	++
	1934-5	1.59	1.41	1.38	0.032	++	.	++
	1935-6	1.59	1.41	1.38	0.032	++	.	++

ment means though not significantly different were in the same order ( $C > N > G$ ) as for the previous seasons.

The observed treatment effect on efficiency index might be brought about either by the *C* plants having a greater percentage of the total dry

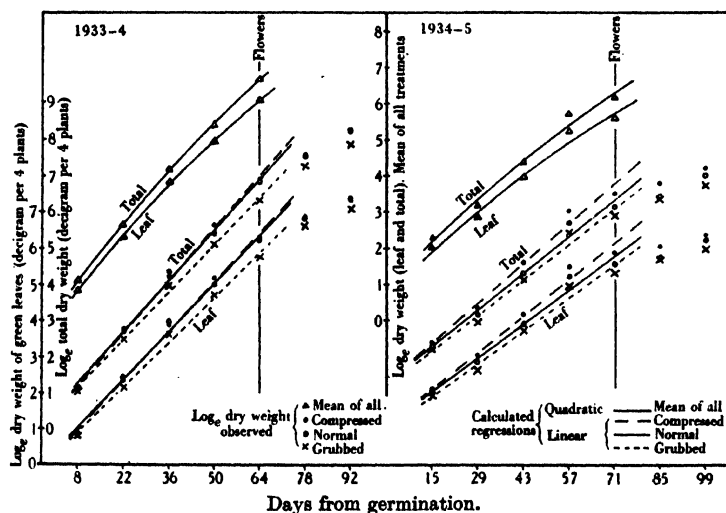


Fig. 4. Regressions of log<sub>e</sub> total dry weight and log<sub>e</sub> dry weight of green leaves on time up to first flowers. Seasons 1933-4 and 1934-5.

weight consisting of leaf, or by their net assimilation rate per unit leaf weight being higher. The treatment differences for dry weight of leaves per cent of total dry weight found in each season are in the opposite order to those found for efficiency index (Table XIII). Therefore the latter must be due entirely to treatment differences in net assimilation rate per unit leaf weight.

In all seasons Compressed had a higher mean dry weight per leaf than Grubbed (Table XIII). The lower weight of leaves per cent of total must therefore have been entirely due to a lower number of leaves per unit total dry weight.

In seasons 1933-4 and 1935-6 the water contents, whether for leaves or whole plants, failed to show any treatment effects. In 1934-5, however, the mean leaf water content over the five occasions up to flowering was lower for Grubbed than for Normal and Compressed (Table XIII). This was confirmed by the similar differences found for mean water content of whole plant (Table XIII). Analysis of covariance for leaf and whole plant water contents shows them to be very highly correlated, and the treatment differences in the former to be entirely explicable in terms of the latter. (Similar high correlations were found in 1936.)

After first flowering in 1934-5 a curious reversal in the order of treatments for water content of whole plant occurred, and for the sixth to the ninth occasions Grubbed and Normal had a higher water content than Compressed. This may have been due to the larger Compressed plants being more affected by drought, and possibly to "ripening off" caused by their larger early crop.

Like the efficiency index, the relative leaf growth rate for each season has a slight downward trend in time (see quadratic regressions, Fig. 4). The treatment effects are also similar; Compressed having a higher relative leaf growth rate than Grubbed for 1933-4 and 1934-5 (Table XIII and Fig. 4), and for 1935-6 the treatment means being in the same order ( $C > N > G$ ) though not significantly different.

Net assimilation rate, on the other hand, is found to have no general trend of rise or fall, up to time of flowering. The fluctuations about the mean rate, which reflect the variation in meteorological conditions, tend to run parallel in the different treatments. As there is no differential effect as between treatments, it is sufficient to compare treatment means over all occasions (Table XIII). In 1933-4 and 1934-5 Compressed had a higher net assimilation rate than Grubbed, while for 1935-6 the treatment means were in the same order ( $C > N > G$ ) though without statistical significance.

It might be suggested that the observed treatment differences in net assimilation were due to differences in area per unit dry weight of the leaves. Examination of the data concerned fails to reveal any such relationship. The higher net assimilation rate per unit leaf weight found for Compressed was therefore presumably due to a higher rate per unit leaf area.

(c) *Lateral roots.*

There seems no reason to suppose that the variability of the root systems would be less than that of the tops, and it would obviously have been impossible to wash out in a satisfactory manner a number of root systems at all comparable with the number of sample plants (tops) taken. In addition to this, the necessary trenches would have spoiled the land for future experimental work. No attempt, therefore, was made to wash out root systems, but some information was obtained in the following manner:

In seasons 1933-4 and 1934-5, if the soil was very moist at the time of sampling, the sample plants were pulled with a slow steady pull instead of being cut. The tap roots were then severed from the stems and marked at 10 and 20 cm. below the ground level. For each sampling unit, the numbers of lateral roots were counted between 0-10 and 10-20 cm. Roots of more than 2 mm. diameter<sup>1</sup> were counted separately. Such larger roots left visible scars if broken off in pulling the plants, and it was therefore possible to increase the accuracy by counting the scars also, which could not be done for the smaller roots. The pulled roots were not weighed, but it was a matter of general observation that the stoutness of the roots corresponded roughly with the size of the shoot, i.e. on the earlier occasions the smaller Grubbed plants had smaller root systems than the larger Compressed and Normal plants. This was confirmed by the early count of laterals in 1933-4 (see below).

1933-4. Root counts were carried out at 78, 127 and 148 days from germination. On the first occasion the large roots (> 2 mm.) showed an average treatment effect over the two depths which only just failed to reach significance. The treatment  $\times$  depth interaction was very significant, and testing the depths separately showed  $C$  and  $N > G$  at 0-10 cm. (Table XIV) but no significant treatment effects at 10-20 cm. Testing the "0-10 minus 10-20" figures for the different treatments showed significant differences in large root distribution (see Table XV). Exactly similar results were obtained for total roots at 78 days (Tables XIV and

<sup>1</sup> Measured with a gauge.



XV) as for large roots, except that the average treatment effect was quite insignificant. The counts of large roots at 127 days and 148 days showed the same type of difference in root distribution, and also in the latter case a significant treatment effect at 0-10 cm. Neither date showed a significant effect of treatment at 10-20 cm.

Table XIV. *Lateral roots per plant, 0-10 cm. depth. Season 1933-4-*

Days	Laterals	Com- pressed	Normal	Grubbed	S.E.	Significant differences		
		<i>C</i>	<i>N</i>	<i>G</i>		<i>C-N</i>	<i>N-G</i>	<i>C-G</i>
78	> 2 mm.	3.85	3.23	1.80	0.349	.	+	++
78	Total	17.78	15.30	12.55	0.813	.	+	++
127	> 2 mm.	5.70	5.85	5.08	0.44	.	.	.
148	> 2 mm.	7.03	7.38	4.73	0.567	.	+	+

Table XV. *Lateral root distribution. Number per plant at 0-10 cm. depth minus number at 10-20 cm. Season 1933-4*

Days	Laterals	Com- pressed	Normal	Grubbed	S.E.	Significant differences		
		<i>C</i>	<i>N</i>	<i>G</i>		<i>C-N</i>	<i>N-G</i>	<i>C-G</i>
78	> 2 mm.	+2.70	+2.10	+0.90	0.360	.	+	++
78	Total	+7.68	+6.05	+0.80	1.272	.	+	++
127	> 2 mm.	+1.85	+1.25	-0.10	0.396	.	+	++
148	> 2 mm.	+1.67	+0.25	-2.48	0.741	.	+	++

The results for 1933-4 show that, at least in the earlier part of the season, the Grubbed plants had smaller root systems than the Compressed and Normal plants. Also that the former had relatively more of their roots at 10-20 cm. and relatively fewer at 0-10 cm.

1934-5. Root counts were carried out at 85 and 113 days from germination, but failed to show any significant effects of treatment or treatment  $\times$  depth interaction. The treatment means for large roots at 85 days were in the same order for each depth ( $C > N > G$ ) as in 1933-4.

### *Final yield*

Fortnightly pickings were carried out on the whole plots in 1935-6 and the middle portions in the other seasons. The treatment effect on total yield over all pickings was insignificant in each season, but a significant treatment  $\times$  occasion interaction was found in 1932-3 and 1934-5. Only the first pickings showed any significance of treatment when tested separately (Table XVI), but in each season except 1936 the last pickings showed the treatments in the reverse order.

Table XVI. *Yields of seed cotton in lb. per acre*

Season	Date	First picking			S.E.	Significant differences			Total yield		
		Com-pressed	Normal	Grubbed							
		C	N	G		C - N	N - G	C - G	C	N	G
1932-3	15. iv. 33	284	332	179	30.4	.	+	+	822	820	732
1933-4	24. v. 34	492	600	515	39.9	.	.	.	775	784	791
1934-5	26. iv. 35	49	37	21	4.7	.	+	++	376	388	254
1935-6	17. vii. 36	69	68	64	9.0	.	.	.	84	91	83

## STATISTICAL ANALYSIS

The significance of the results presented in the preceding sections has in all cases been tested by the method of analysis of variance (10).

In carrying out the plant sampling, two or more independent "sampling units" were taken from each plot on each occasion. The analysis of variance for primary data from these samplings includes on each occasion an estimate of the "sampling variance" due to variation between sampling units within plots. From this sampling variance and the between-plot variance, the loss of information due to sampling has been calculated by the method of Yates & Zecopanay (25). In 1933-4 the size of "sample" (i.e. the aggregate of sampling units from one plot) expressed as a percentage of a full stand over the area sampled was 3.6 per cent before thinning (45 days from germination) and 1.8 per cent after. The corresponding figures for 1934-5 are 5.3 and 2.7 per cent. In 1935-6 the sample on the first occasion was 9.1 per cent and on the second 13.6 per cent of the plot. The estimated loss of information due to sampling was in general about 30-40 per cent for one occasion, but the efficiency would be much greater for a smooth curve fitted over a number of occasions.

In calculating the derived data from plant samplings, plot means have been used and not the figures from individual sampling units. Analysis of variance on these plot means has in all cases included several occasions, and for some data the degrees of freedom for occasion have been split up into those for linear regression of the measure ( $y$ ) on time and for deviation from linear regression. In all cases the appropriate interaction with blocks ( $B$ ) has been used as error. Thus treatment ( $T$ ) variance is tested against the  $T \times B$  interaction, occasion ( $O$ ) against  $O \times B$  and the interaction  $T \times O^1$  against the triple interaction  $T \times O \times B$ . The similar comparison of the linear regression portion  $O'$  of the occasion variance with

<sup>1</sup> Where  $T \times O$  has been found significant, analysis of variance has also been carried out separately for the different occasions.

$O' \times B$  as error, or of  $T \times O'$  with  $T \times O' \times B$  is valid, since it merely amounts to an analysis of variance of the values ( $Y$ ) calculated from the linear regression for each plot. The comparison of the deviation portion  $O''$  with  $O'' \times B$  or of  $T \times O''$  with  $T \times O'' \times B$  is similarly equivalent to analysis of variance on the deviations ( $Y-y$ ) of the observed values from those calculated from the linear regressions. The "deviation from the linear regression" degrees of freedom, with their corresponding sums of squares, can be split up further into the separate higher order regression terms and similarly tested. This has been done in the case of the quadratic terms for  $\log_e$  (total dry weight) and  $\log_e$  (leaf dry weight).

For soil data (plot means) and for plot yields analysis has also been carried out including several occasions, but the regression terms have not been calculated. The same applies to soil data and root counts including several depths on single occasions.

#### GENERAL DISCUSSION

The different cultivation treatments applied before sowing have been shown to have produced effects on the consolidation of the ploughed soil, persistent at least during the earlier parts of the seasons. For 1935-6 a treatment effect on density was directly demonstrated, and it is most probable that in the previous seasons the observed treatment differences in consolidation (resistance measurements) were also largely due to differences in soil density.

Treatment differences in moisture content per cent of dry weight of the ploughed soil were on the whole small, although frequently significant. As has been pointed out, even when the Grubbed treatment was found to have a higher moisture content per unit dry weight, it might yet have a lower one per unit volume by reason of its less dense soil. When the Grubbed soil was found to have less moisture per unit dry weight, after rains in 1935-6, the effect must have been even greater in terms of volume. A similar argument is applicable to the nitrate contents which in three seasons showed no significant treatment differences in terms of weight. Even if all the treatments had equal nitrate contents in the ploughed soil in terms of weight, the Compressed treatment must have had more nitrate than Grubbed in terms of volume of soil, and this view is further supported by the almost significant treatment effect on a weight basis found on one occasion. It is therefore probable, though not proven, that unit volume of the ploughed soil contained more nitrate on Compressed than on Grubbed.

As far as water supply or nitrate supply are concerned, therefore, there is no *direct* evidence to show that the cultivation treatment resulted in any large differences in these respects. The only significant effects noted indicate a higher water supply in the Grubbed soil (p. 521) but this was associated with reduced growth and early yield. Season 1935-6 was exceptional in showing a lower water content in the Grubbed treatment (p. 521), again associated with reduced growth. The lower internode lengths and smaller leaves of the plants on the Grubbed soil do in fact provide evidence of a more restricted water supply (Crowther (4)). The variation in density as has been suggested might result in a lower content in the Grubbed soil on a volume basis and therefore the effect of nitrogen and water as determining the results cannot be dismissed. Indeed the higher relative leaf growth rate in the Compressed as compared with the Grubbed treatment indicates a higher nitrogen supply, since, as Crowther and Gregory have stressed, nitrogen supply is the controlling factor in this respect. This is confirmed by the same differences noted between these treatments in node production and flower production.

Accepting as a provisional hypothesis a higher nitrogen supply in the Compressed soil, this might result from the better drainage of the Grubbed soil (p. 523) allowing nitrate to be washed to greater depths by rain. The root distribution in 1933-4 does not conflict with the view that nitrogen supply is differentially affected by leaching, since in the Grubbed soil relatively more roots were produced at lower depths (Table XV). In season 1934-5, however, the reverse result was found, the differences of root production in the zones 0-10 and 10-20 cm. showing in all treatments larger root production near the surface and the effect being greatest in the Grubbed treatment ("total roots":  $C + 18.2$ ;  $G + 34.6$ ). These results are not statistically significant. In view of the suggested importance of leaching as controlling nitrogen supply, it should be noted that season 1933-4 was comparatively dry whereas 1934-5 was very wet. In the wetter season therefore when more washing out of nitrate would be expected there is no evidence of a deeper root system in the Grubbed soil, rather the reverse.

Nitrogen supply to the plant eventually depends on the concentration of soluble nitrogen in the soil solution, and therefore on the difference between rate of production of nitrate by nitrification and removal by leaching, root absorption and use by other soil organisms. It is generally believed that production of nitrate depends on efficient aeration, and it is in this connexion that the results of these experiments afford interesting evidence. The consolidation of the soil in the Compressed as contrasted

with the Grubbed treatment must very considerably lower the rate of supply of oxygen in the former. The presumably poorer aeration is, however, in all seasons associated with better growth. It may be doubted, therefore, whether aeration is so directly related to plant development as Howard (18) has supposed. In tropical soils it is a matter of common experience that opening up the soil leads to a rapid disappearance of organic matter through the activity of micro-organisms. For such activity consumption of nitrogen is necessary, and it is possible that the Grubbed treatment in admitting more oxygen has reduced the available nitrate to the plant by allowing the competitive action of the microflora to remove in advance available nitrogen supply. From these considerations it would appear that the "control" treatment in these experiments should not have been "normal" cultivation but no cultivation at all, apart from the hoeing necessary to remove the previous crop and weeds.

The emphasis has all along been laid on the relations of nitrogen in controlling growth. The lower assimilation rate noted in the plants on Grubbed soil may also be due to a lower level of nitrogen supply. Thus Müller (22) claims to have found just such an effect of nitrogen deficiency with *Sinapis alba*. Crowther (4) on the other hand confirms Gregory (12) and Gregory & Richards (14) in finding no effect on net assimilation rate up to flowering stage. Since in the experiments of Crowther the cotton plant was investigated and nitrogen level was deliberately varied, the results cited have a direct bearing on the present problem.

The lower assimilation rate on the Grubbed treatment may have been due to relative deficiency in other nutrients. The soil at Barberton is known to be deficient in phosphorus and potash. Large responses to phosphorus addition are always obtained, and a lesser but very significant response to potash addition has also been found in recent years. As in the experiments under review a liberal dressing of phosphorus was given it is unlikely that phosphorus supply is in question. No additional potash, however, was given. It is well known that potash deficiency lowers the assimilation rate per unit area (Briggs (3), Maskell (21), Gregory & Richards (14), James (19), Richards (23)), while Gregory & Richards (14) show for barley that at the same time it increases the rate of respiration. It must therefore lower the net assimilation rate. In absence of analytical data of potash content of the plants, the relative deficiency of potash in the Grubbed soil remains only as a suggestion. The effect of the density of the soil in affecting nitrogen supply on a volume basis holds also for potash.

The net assimilation rate is an average measure of the assimilatory

activity of all the leaves on the plant. The senescence of the leaves is associated with a fall in assimilation rate and if, therefore, the ageing of the leaves was more rapid on the Grubbed soil the net assimilation would show the difference noted. As senescence is related directly to nitrogen supply it is possible that the explanation lies here. In absence of data on the percentage of dead leaves in the different treatments this suggestion loses much of its cogency. The higher percentage leaf weight found on the Grubbed treatment is opposed to such a view.

One further suggestion may be put forward. In season 1934-5 the water content of the plants on the Compressed soil was significantly higher. Whether this affected in any way the behaviour of the stomata, leading to more ample carbon dioxide supply, is only conjectural, especially in the absence of significant correlation between net assimilation rate and leaf water content.

It must be admitted that the cause of the more rapid growth due to compacting the soil cannot be satisfactorily accounted for. The important point, however, is the very definite establishment of this effect. Clearly its elucidation will demand a more rigorous physiological analysis than has been possible in these experiments.

#### SUMMARY

1. Experiments were carried out in four seasons at Barberton, South Africa, occupying approximately  $2\frac{1}{4}$  acres of ground. Three soil treatments were used, viz. Normal (N), Compressed (C) and Grubbed (loosened) (G).

2. Measures of consolidation of the soil were made with a specially designed apparatus, and large differences established (pp. 516-520).

3. Estimations of water content and nitrate content were carried out. No great differences were found (pp. 520-524).

4. Fortnightly samples of the plants were taken, from which numerical data were collected designed to estimate morphological development (primary data, pp. 526-529).

5. Derived data on net assimilation rate, relative leaf-growth rate and efficiency index were calculated (pp. 529-533).

6. Growth in total dry weight was found to conform approximately with an exponential law up to the time of flowering. Up to this stage also net assimilation rate was found to remain constant.

7. Growth differences between treatments were related to variation in net assimilation rate only (p. 531).

8. The results were analysed statistically. Significant results alone are emphasized.

9. In all respects growth and development on the consolidated soil was more rapid than that on the grubbed soil, normal being generally intermediate.

10. The results are discussed with reference to the question of nitrogen supply, water supply and aeration of the soil.

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# COMPRESSIBILITY CURVES AS A QUANTITATIVE MEASURE OF SOIL TILTH

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(With Plates VIII and IX and Four Text-Figures)

## INTRODUCTION

THERE is at present no quantitative measure of soil tilth. If the farmer is asked to express an opinion on the tilth of a field, he generally presses the soil with his foot and notes the compressibility, the ease with which the lumps of soil disintegrate, the stickiness, and possibly the tendency to recoil elastically when his weight has been removed from the soil. These properties are judged at a compressive stress considerably less than that obtaining in normal cultivation processes. Thus a man weighing 150 lb. if he puts the whole of his weight on his one foot, assuming the area of contact to be 35 cm.<sup>2</sup>,<sup>1</sup> will exert a stress on the soil of less than 200 g./cm.<sup>2</sup> whereas, in practice, he seldom shifts more than half his weight on to the foot with which he is testing, so that a stress of the order of only 100 g./cm.<sup>2</sup> is probably applied. Ballu<sup>(1)</sup> calculates that an ordinary farm horse exerts, under its own weight, compressive stresses of the order of 2000–4000 g./cm.<sup>2</sup> if the ground is hard enough for the whole weight to be taken by the shoes, that tractor tyres produce a stress of 1000–3000 g./cm.<sup>2</sup>, and caterpillar wheels of the order of 250 g./cm.<sup>2</sup>, though these stresses are only exerted for very short periods of time. The stresses produced on the mouldboard of the plough are very variable. Nichols<sup>(2)</sup> has worked over a stress range from about 350 to 2000 g./cm.<sup>2</sup>, and points out that the lower part of this range, although more complex than the upper part, is, “from a practical point of view . . . quite important, as pressures are far above the average pressure exerted by the plow”. Nichols worked on soil carefully prepared in “a fluffy finely divided state, without the formation of lumps or puddled particles”.<sup>3</sup> Work by other authors has generally been confined to soil removed from its natural environment.

In designing an apparatus to measure quantitatively and imper-

<sup>1</sup> Estimated by observing the “wear” on a pair of old rubber boots.

<sup>2</sup> For a complete account of the development of cultivation processes see Keen<sup>(3)</sup>.



sonally what the farmer gauges by his skill and experience, it is advisable that the weight to be applied to the soil should be considerably larger in area than any soil lumps likely to be encountered. If stresses of the order of  $1000 \text{ g./cm.}^2$  are to be applied, this would involve the transportation and manipulation of many hundredweights of metal, and in practice a compromise must be reached, both stresses and areas being smaller than those theoretically most desirable.

#### THE FIELD APPARATUS

For this purpose the apparatus shown in Pl. VIII was constructed. The weight consists of four cylinders of iron *A*, diameter 15 in., laid one above the other, each weighing about  $\frac{1}{2}$  cwt., the whole weight being hung from a point just above its centre of gravity, so that unevennesses in the soil surface only produce very small restoring forces. This weight is hung from a spring balance *B*, which can be raised or lowered by a windlass *C* operating through a worm, the whole being supported by a tripod fastened by iron pins to a triangle of iron resting on the soil surface. At each corner of this triangle is brazed a circular disk, 8 in. in diameter, to prevent the base from sinking into the soil. This apparatus can be placed in position without any disturbance of the soil beneath its centre, over which the weight initially hangs. The weight is lowered until its surface just touches the topmost summits of the lumps of the soil surface. A duralumin rod *D* is pivoted to the suspension between the spring balance and the weight, and is suspended on a hardened steel knife-edge attached to an independent iron rod *E* bent at right angles at both ends so as to penetrate the soil, and likewise fitted with 8-in. disks. The further end of the duralumin rod is ground to a point, which is trained on to a vertical millimetre scale held by another independently "disked" iron stand *F*.

The weight (approximately 230 lb.) is lowered on to the soil by stages; the effective load on the soil is thus 230 lb. minus the spring-balance reading. The increments of load are applied at  $\frac{1}{4}$ -min. intervals, and, immediately before each increment, the reading, *L*, of the spring balance and  $\sigma$ , that on the deformation scale, are recorded. The increments of load are made as nearly equal as possible. Two operators are required for the tests. The principal operator calls the  $\frac{1}{4}$ -min. intervals from a stop watch, reads the  $\sigma$ -scale, and records all the data, while an assistant gives a turn to the windlass when instructed, and calls out the spring-balance readings.

Interpretation of the significance of the data obtained will be largely reserved for a later section, but it will be advantageous at this stage to

examine a single experimental curve, such as that shown in Fig. 1. As the load is increased, the deformation increases at first fairly rapidly, and

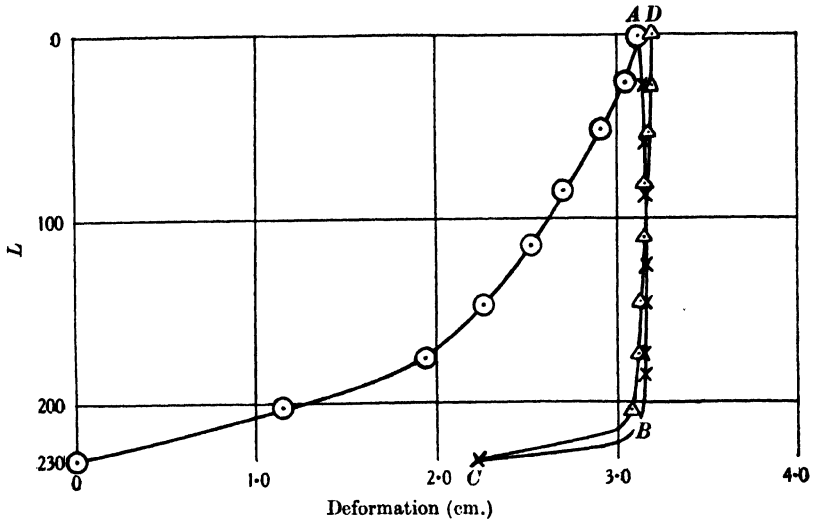


Fig. 1. Load-deformation curve for a field soil.

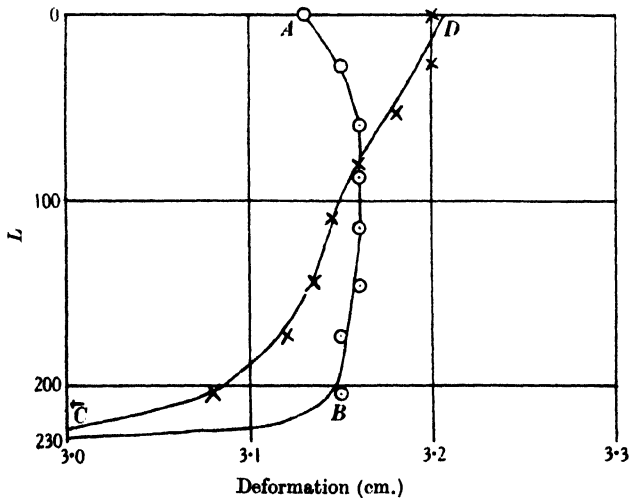


Fig. 1 a. Replotting of data given in Fig. 1 to show elasticity effects.

later more slowly. By the time the whole load is resting on the soil, a total compression *A* has been produced (circles on Fig. 1). This is partly elastic (recoverable) and partly plastic (non-recoverable) (*vide* Schofield &

Blair (4); Nádai (5). If the load is removed from the soil by stages at the same rate as it was applied (crosses in Fig. 1), the soil surface actually rises very slightly. That this effect is real has been checked by doing tests on hard incompressible surfaces. These tests show that any elastic "give" in the apparatus itself must be exceedingly small. The loading curve, *OA*, is complex in shape, and its form will be considered later. It is by no means invariably of the form shown in the figure, but in all soils investigated the permanent plastic deformation is large compared with the recoverable elastic deformation shown in the unloading arm of the curve *AC*. The tailing off of the curve from *B* to *C* is apparent rather than real, and depends simply on the unevenness of the soil surface and consequent difficulty in assessing a correct zero. In Fig. 1*a*, the scale has been increased so as to magnify the hysteresis loop. The amount of flow which takes place during the unloading and subsequent second loading of the soil (triangles in Fig. 1, crosses in 1*a*) is largely determined by the moisture content, whereas the steepness and shape of the first loading curve depend more on the looseness of tilth of the soil. These latter factors may be subdivided into (*a*) flow and rupture properties of individual soil lumps or crumbs, and (*b*) capacity of these lumps to alter their packing under load. Although the elastic properties of the soil in tilth are interesting, the recoverable deformations are so small (of the order of a millimetre in the experiments shown) that their practical interest is not so immediate as that of the plastic deformations. Nichols (2) has pointed out the difficulties involved in fitting any equation to the part of the compression curve when stresses are relatively low, and before attempting any complete treatment to actual experimental results it seemed advisable to evolve some method of plotting the data which should give a straight line as the ideal case, divergencies from linearity then being treated as a measure of abnormality of one sort or another.

#### THE "IDEAL" RELATIONSHIP BETWEEN STRESS AND DEFORMATION IN COMPRESSION OF A CRUMB-STRUCTURED MATERIAL

This problem has been touched on by Terzaghi (6) and treated much more completely by Pokrowski & Bulytschew (7). These latter authors point out that in compression, the soil particles become increasingly disturbed out of their original structural formations and suggest an equation in which the stress gradient  $dS/d\sigma$  is proportional to the stress at any point on the loading curve, multiplied by the difference between this stress and the limiting stress at which the disturbance of structure is

complete. For small stresses, this equation reduces to  $dS/d\sigma \propto S$ , in which limiting case straight lines should be obtained by plotting  $\log S$  against  $\sigma$ . This treatment has been applied to some of the data from an experiment described in a later section, and the curves, which show a fair linearity, except in cases where the soil is very incompressible (1), are given in Fig. 2. The numbers refer to the treatments described later.

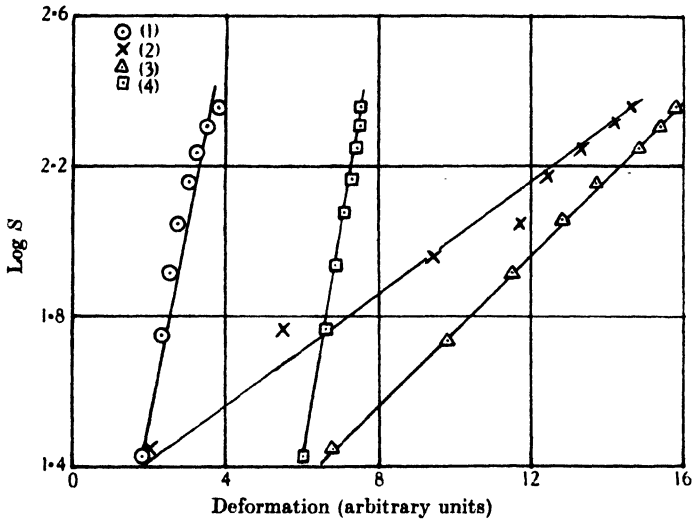


Fig. 2. Data from field soil compression tests plotted according to simplified Pokrowski equation.

It would seem more logical, however, to relate the stress gradient to deformation rather than to stress. The change in structure on compressing the soil may be regarded as a type of work-hardening and certainly depends more on the amount of compression than on the stress, and the total load exerted on the weight by the soil will rise proportionally to the area of contact as the area increases with the sinking of the weight. At first, the area of contact will increase rapidly, whereas repacking and shear effects will be slight. As compression proceeds a region will be reached in which the two processes will have about an equal importance, and here the stress might be supposed to vary ideally with the square of the deformation—varying directly with the deformation for each of the two factors, increased surface of contact and packing and shearing.<sup>1</sup> This is equivalent to assuming that  $dS/d\sigma \propto \sigma$  for a crumb-structured material.

<sup>1</sup> The shearing properties of the soil are being very thoroughly investigated by Pigulevski and his colleagues (8).

For an elastic solid<sup>1</sup>  $dS/d\sigma = \text{constant}$ , or  $S$  increases proportionally to  $\sigma$ , and an approximation to this condition occurs for very hard dry soils, or at the top of the stress-strain curve, when compacting is considerable. (Experimental evidence for this will be given in a later section.) For a soil the loading curve is thus sigmoid in shape, for, in the lowest stress region, the stress varies as a power of the deformation greater than two, and at the high stress range it varies more nearly as  $\sigma$ . The intermediate region is that in which the repacking, shear, and rupture of the soil crumbs are principally taking place, and this region, where  $S$  varies approximately as  $\sigma^2$ , is in general the more marked the better the tilth of the soil.

It is clear that a very great many data will have to be examined before it can be established as a certainty that this treatment, which is partly empirical, gives the nearest approximation to agreement with the experimental figures. As Nichols rightly says, no true soil will conform exactly to any simple equation. A simple flow equation presupposes that the fine structure of the material is of such an order that statistical laws can be applied. The laws of flow for a true fluid depend on the great number and small size of the shearing units. In the flow of pastes complications arise due to size and shape of the shearing units (9) and, in natural soil, where these become of the same order of magnitude as the apparatus, individual particles may show their effects on the curves (*vide infra*). Before any information can be obtained as to the individual eccentricities of particular samples, the curves must be reduced to a form where the gross effects of compression have been as far as possible reduced to order. For this reason it seemed good to design an apparatus in which curves could be obtained, giving the deformations plotted against the square root of the stress, the deformation being given at a constant rate. Partly because such an apparatus would be difficult to construct on a field scale, and partly because it was desired to investigate conditions of tilth, some of which could not be conveniently obtained on the farm, the apparatus was made for use in the laboratory. The technique is subject to the criticism applicable to all laboratory tests that the soil is liable to some changes in condition in the process of transferring to the laboratory, however carefully the operation is done. The problems are therefore being studied at the same time by both methods: first, the field soil loading apparatus already described in which loads are applied as far as possible in equal increments after equal intervals of time, the rate of deformation being increased and elastic as well as plastic deformations being considered;

<sup>1</sup> For a true fluid, the stress would be proportional to the *rate* of deformation and independent of the absolute deformation, so that  $dS/d\sigma$  would be zero.

and secondly, the laboratory method, in which deformations are given at a carefully controlled constant rate, the square root of the stress built up being automatically plotted against deformation. In this latter method, elastic hysteresis phenomena are not studied.

#### THE LABORATORY APPARATUS<sup>1</sup>

The apparatus is shown in Pl. IX. *A* is an enamelled metal tray ( $20 \times 15$  cm.) containing a layer of soil 2.5 cm. deep. (The effect of depth of layer has been investigated and the depth here quoted has been found satisfactory.) This tray is hung by four chains and counterpoised by a bucket *B*, containing water. A constant speed motor *C*, operating through a suitable worm gearing, causes the tray to rise at a constant and very slow rate of about 2.35 mm./min. A lead weight *D* (= 1670 g.) is hung from the beam *E* of a counterpoised balance resting on knife edges *F*. The weight is a cylinder of diameter 6.0 cm. It is hung from a point just above its centre of gravity, the suspension passing upward through a wide enough hole to allow for a maximum of about  $10^\circ$  of tilt if the surface of the soil is uneven. The force tending to right the weight is extremely small. Except at the lower end, the suspension is of steel wire to avoid errors due to elasticity in the suspending thread.

As the rising soil surface tends to take up the load of the weight, the beam of the balance rises, thereby opening a valve *G* which allows mercury, stored in the container *H* and kept at a constant head by adjustment of the tap *J*, to run into the bucket *K* which is hung on the same arm of the balance as *D*. This compensates for the change in load produced by the gradual lifting of *D*. The bucket *K* has two of its sides parallel and two sloping, so that the height of the mercury collected is proportional to the square root of its mass, and hence to the square root of the load pressing on to the soil. The bottom of *K* is made flat, and before each test, 2.5 c.c. of mercury are run in from the burette *L* to cover this flat bottom. On this layer of mercury floats a small steel weight attached by means of a cotton passing over pulleys to a pen *M*, the other end of whose holder is again attached to a smaller counterpoise weight *N*. As the mercury lifts the weight in *K*, *N* pulls the pen *M* across the paper which is attached to a glass sheet by two rubber bands. The glass sheet is driven in a direction at right angles to the movement

<sup>1</sup> This apparatus was described, with special reference to its application to soil amelioration problems, at the Conference of the Sixth Commission of the International Society of Soil Science, held at Zürich, August 1937. The author is indebted to Mr D. Morland for much help in the construction of the apparatus.

of  $M$  by a second gearing from the motor  $C$ , and as  $D$  does not move appreciably  $M$  traces a curve whose ordinate is proportional to the square root of the load on the soil, and whose abscissa is proportional to the amount of deformation. The two axes are drawn, one by raising and lowering  $N$  before the test, and the other by the second pen  $O$  attached rigidly to the frame, which is aligned so that the two lines so produced are at right angles. The total load represented in the diagrams amounts to 59 g./cm.<sup>2</sup>, though, since only a part of the surface of the weight is in contact with the soil during much of the run, much higher local stresses must be produced. Work with a larger weight has also been carried out, but it is difficult to prevent weights giving high loads per cm.<sup>2</sup> from becoming unduly top-heavy. Once the motor has been started, it will be observed that the whole process, including the drawing of the curves, is automatic, except only for the adjustment of the tap  $J$ , which is a matter of secondary importance.

#### *Data obtained from laboratory apparatus*

A number of curves obtained with this apparatus are given in Fig. 3: (1) is that for a dry sand, (2) for a wet sand in such a condition that the material coheres into a loose kind of structure, and (3) the same sand wetted to such an extent that the structure again disappeared. It is clear that in the two cases in which there is no crumb structure, the curve is concave to the deformation axis throughout, and calculation shows that the stress varies approximately as the deformation, whereas where there is a structure the curve is predominantly convex, the strain varying with some power of the stress higher than 2. Intermediately, approximately straight-line curves may be obtained. A "step-ladder" formation just visible at the lower end of curve 2 indicates the disintegration of individual crumbs. In Fig. 4a values of  $S$  calculated from  $\sqrt{S}$  readings read off the dry-sand curve are plotted against  $\sigma$ . It is clear that under the circumstances of this test the "elastic" law is approximately obeyed.<sup>1</sup> For comparison, curve 4 (Fig. 3) shows a test made on an ordinary rubber sponge. This is more or less elastic, as is shown from the  $S/\sigma$  curve (Fig. 4b). The modulus is not quite constant for low stresses due to peculiar surface properties.

A curve for a wet, structureless soil is shown in Fig. 3, 5, and may be

<sup>1</sup> The laboratory apparatus is not designed to study elastic phenomena, and it is known that there is some "give" in the apparatus itself. For this reason reliable elasticity moduli could not be calculated from these curves.

compared with that for a soil in fairly good tilth (Fig. 3, 6). The significance of the differences in form will be discussed later.

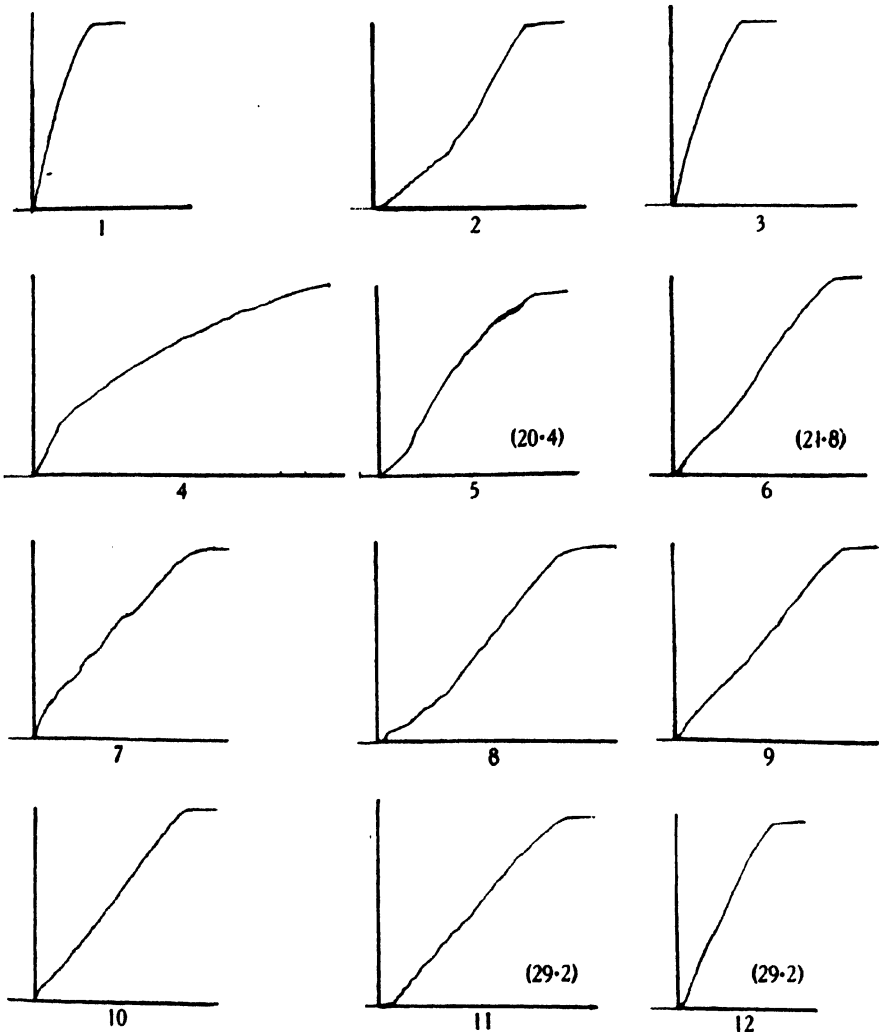


Fig. 3. Compressibility curves from self-recording laboratory apparatus. Ordinate is square root of the load, and abscissa is deformation. Moisture contents are given as numbers in brackets.

It is natural to enquire how far the size of lumps of soil affects the shape of the curve. In order to study this point, the soil used for Fig. 3, 6 was sieved into a series of fractions, curves for the individual fractions



being taken. These are shown in Fig. 3, 7 particles  $> \frac{1}{2}$  in., 8,  $\frac{1}{2} - \frac{1}{4}$  in., 9,  $\frac{1}{4} - \frac{1}{10}$  in., and 10,  $< \frac{1}{10}$  in. The differences are surprisingly slight.

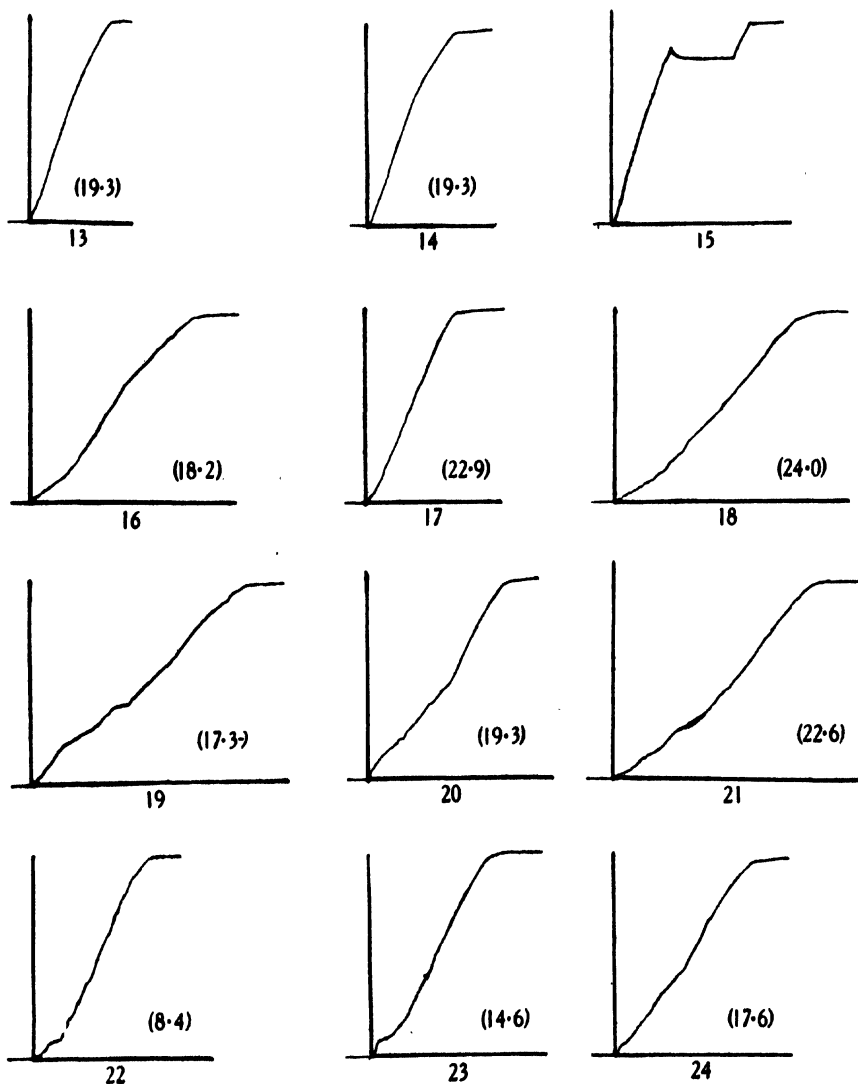


Fig. 3. (Continued).

Except in the case of the smallest fraction, there is a general tendency for the total compressibility to increase with decreasing size of particle, and the biggest particles not only tend to give a somewhat erratic curve, but

the stress clearly varies as some power of the deformation less than 2. This leads to the question as to how far the compression observed is due to shear or crushing of individual lumps, and how far to packing effects. Although local stresses must sometimes be much higher than the mean value of 59 g./cm.<sup>2</sup>, not very many crumbs are actually crushed, except in the case of soils having artificially prepared very soft crumbs. We are mostly concerned with the distribution of deformation between shear and repacking. Mr G. H. Cashen suggested that experiments might be done on the compression of three crumbs chosen as far as possible to be of the

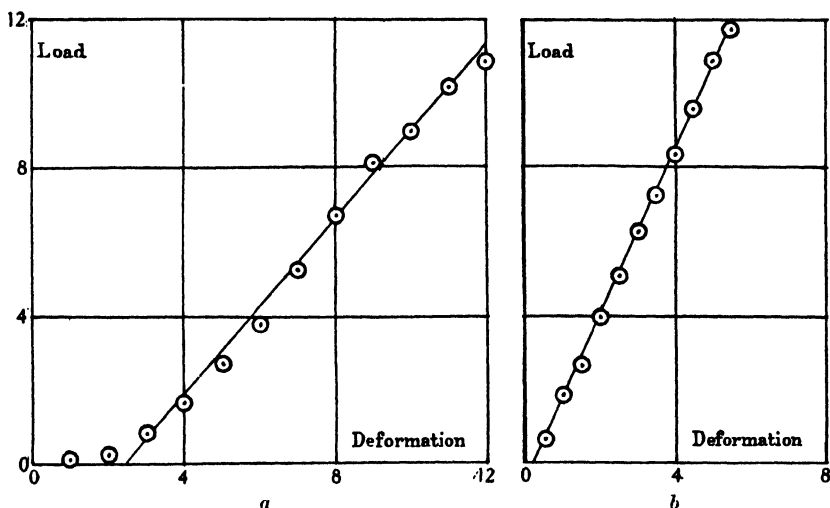


Fig. 4. Compressibility curves plotting deformation against load directly. *a*. Dry sand (calculated from Fig. 3, 1). *b*. Rubber sponge (from Fig. 3, 4).

same size (about 2 cm. diameter), and that comparison should be made with the curves for the complete soil from which the crumbs were selected. The results of these experiments are shown in Fig. 3, 11–15. Fig. 3, 11, is a curve for a surface soil obtained from a wood where the soil condition was kept good by natural processes. The soil lumps were small, and three of the largest of them had to be selected to obtain curve (12). Curve 13 is for a complete soil from the same neighbourhood, but taken from a nearby waterlogged cultivated field which had not been ploughed for some time. The soils had both been somewhat dried out in the laboratory before testing, and the latter soil had set into large hard lumps, three of the smallest of which had to be selected for the test 14. It is clear that the compressibility of the larger lumps from the good soil and that of

the smaller lumps from the bad soil were very similar, though in the former case this compressibility represented only a small fraction of the total compressibility of the soil, whereas in the latter, repacking can have played hardly any part in the building up of the composite curve. In none of these three-crumbs tests did the crumbs crush completely. In order to show the effect of such a collapse on the curve, a soil made into very brittle crumbs in the laboratory was selected, and three crumbs tested. The curve is numbered 15. The apparent fall in stress immediately after the breaking of one of the crumbs (the only complete break during the test) is due to an upsetting of the surface tension conditions round the weight floating on the mercury surface.

#### EFFECT OF CHANGES OF TILTH ON THE CURVES

If the line of argument followed in this paper has been cogent, it should be possible to follow changes in tilth produced by natural processes by means of the laboratory technique described. Certain effects, such as slight surface "capping", may be incapable of preservation during the process of transferring the soil from the field to the testing tray. If these effects are to be studied the field technique must be used; but many of the changes produced by climatic or cultural processes on the soil-crumbs structure will readily survive transportation. The effect of a spell of frosty weather is shown in Fig. 3, 16-21. Curves 16-18 are for the samples taken from three locations before the frost: (1) the top of a furrow on ploughed land in fair tilth, though over-wet, (2) a nearby depression where the lack of drainage had produced a really bad condition, (3) an allotment whose soil had been well cared for and suffered only from excessive moisture. (Moisture figures are given in brackets on the figure, and refer to the percentage moisture on a wet basis determined by drying the soil at 110° C. for 24 hours.)

Following a few days of frost, further samples were taken from the same three places. A considerable improvement in tilth is shown in the case of the soil from the top of the furrow, a greater retentive improvement for the waterlogged sample, but little change is found for the soil already in good condition (curves 19, 20 and 21 respectively). A test designed to demonstrate the effect of freezing in the laboratory on a really good garden soil gave a completely negative result, the only changes produced in the curve being explainable by the slight drying out.

Although space considerations preclude the publication of all the curves, the above soils were all tested not only soon after being brought

to the laboratory, but frequently for a period of some days during the drying-out process. After each test the soil was dug carefully to restore the uncompressed condition of the surface and, although the continuous pressing and digging is bound in the long run to affect the course of the drying process, experiments repeated immediately after such treatment agree very closely with the initial tests. It is therefore believed that the results of following such a drying out in this way are of interest. Three curves obtained after considerable drying are shown in Fig. 3, 22-24, which were obtained from the corresponding dried samples used for 19-21. During the same period of time (about 4 days) the better field soil had dried most, the waterlogged soil, on account of its bad structure, had dried least, and the good garden soil intermediately, due, no doubt, to the very large amount of organic matter which it contained. The decrease in total compressibility is clearly marked in all three cases. The shape of the curves has not been greatly affected, except for a slightly increased step-ladder structure in the case of Nos. 22 and 23 where somewhat hard intractable lumps are formed when the soil is dried.

Sticky points and lower plastic limits were determined (Atterberg) on many of these soils, and it was observed that the latter, which is known to correspond reasonably closely to that moisture most suitable for cultivation, also in many cases corresponds approximately to the point at which a well-marked step-ladder formation is observed in the compression curves. Such conclusions must, however, be treated with caution, since the moisture is often not very evenly distributed throughout the soil mass, and a small number of large lumps having a moisture content differing from the mean for the whole sample may affect the fine structure of the curve quite appreciably.

#### CONCLUSIONS ON INTERPRETATION OF CURVES FOR EVALUATION OF TILTH, AND APPLICATION TO FIELD EXPERIMENTS

The process of compression is not yet sufficiently understood for a full and complete interpretation of the curves in terms of tilth to be possible. In the field experiments, it is not known how the stresses in the soil vary with depth, though preliminary experiments (conducted in co-operation with Mr Cashen) in which closed rubber tubes attached to manometers were buried at different depths in the soil, indicate that although there is probably a time-lag, the compression effect goes down at least as far as the soil has been cultivated. It is intended to extend these experiments and to publish the results in a later paper. In the laboratory technique

the situation is somewhat different. Here, the effect of depth of soil layer is surprisingly slight, and the deformations are partially restricted by the proximity of the sides of the tray.

Even before the laboratory apparatus had been designed, interesting semi-quantitative results had been obtained in the field by testing areas within a small space which had been (1) dug once and rolled, (2) dug once not rolled, (3) dug twice and not rolled, and (4) dug twice and rolled. Tests were done at different spots on these plots chosen in a random manner, and the total compressibilities (arbitrary units), taken from the best straight line on a  $\sqrt{S}/\sigma$  basis to eliminate surface unevenness effects, were as follows:

Treatment	Days after cultivation treatment				
	0	6	11	21	38
1	2.7	4.4	5.5	6.5	4.7
2	14.2 (?)	11.1 (?)	21.5	18.1	15.6
3	11.6	9.0	15.9	16.1	16.3
4	1.9	2.5	3.7	3.7	6.7

(Figures marked (?) were not as accurate as could be desired.)

The first of these experiments (0 days) provides the data used to test the validity of the  $(\log S)/\sigma$  equation, and shown in Fig. 2. Only the first loading figures are given. The complete data for No. 3 are those used to show, in Figs. 1 and 1a, the general shape of the curves. A number of other factors as well as total compressibility were considered, and certain regular effects noted, but it seems wiser to confine our attention at this stage to the broadest outlines, since the experiments have not yet been repeated, and it is hoped to undertake further field experiments, which, from the experience already gained, should be of a higher order of accuracy.

It is clear from the above table that, for all treatments, the soils have become more and not less compressible during the first few weeks of digging, an effect probably due to an increase in moist content from 16 to 26 per cent. When the soil has been rolled, this "lifting" effect is very marked. Digging the soil twice in succession has not made it any more but rather less compressible, whether the soil is afterwards rolled or not.

These preliminary results indicate the kind of information which such experiments should give. The following interpretation of the different characteristics of the curves will serve as a working hypothesis, and may be followed with reference to Fig. 3:

(1) Soils in good tilth show a long deformation range in which the  $\sqrt{S}/\sigma$  curves are approximately linear. Upward curvature (increasing

$d(\sqrt{S}/d\sigma)$  is preferable to the reverse, and a big total compressibility is generally a sign of good condition.

(2) A long initial range where  $d(\sqrt{S})/d\sigma$  is low, especially if the curve is irregular, indicates an uneven surface of rather intractable lumps.

(3) Very wet or very dry soils give curves concave to the deformation axis for most or all of their lengths. The latter usually give big step-ladder effects. A very light powdery soil may give a fairly high compressibility, but the curve is invariably concave.

(4) Some step-ladder effect is advantageous—a perfectly smooth curve indicates a poor structure.

(5) All data are best interpreted in the light of the moisture content of the soil when tested in relation to its Atterberg constants.

These conclusions are derived largely from experience in the laboratory tests. It remains to be seen how far the differences in the method of stress application will cause them to require modification before application to the field data.

#### SUMMARY

1. A preliminary account is given of experiments on the compressibility of soils in field condition, and two methods for obtaining compressibility curves, one for the field and one for the laboratory are described. The laboratory apparatus automatically draws a curve relating deformation to the square root of the load built up.

2. The theoretical relationship between load and deformation is discussed, the conclusions reached being at this stage semi-quantitative.

3. Laboratory compression curves are shown to indicate the characteristics of soils in various states of tilth, and the effects of drainage condition, frost action, etc. are discussed.

4. Such factors as size of soil crumb, depth of layer tested, and moisture content of soil samples for laboratory studies are considered.

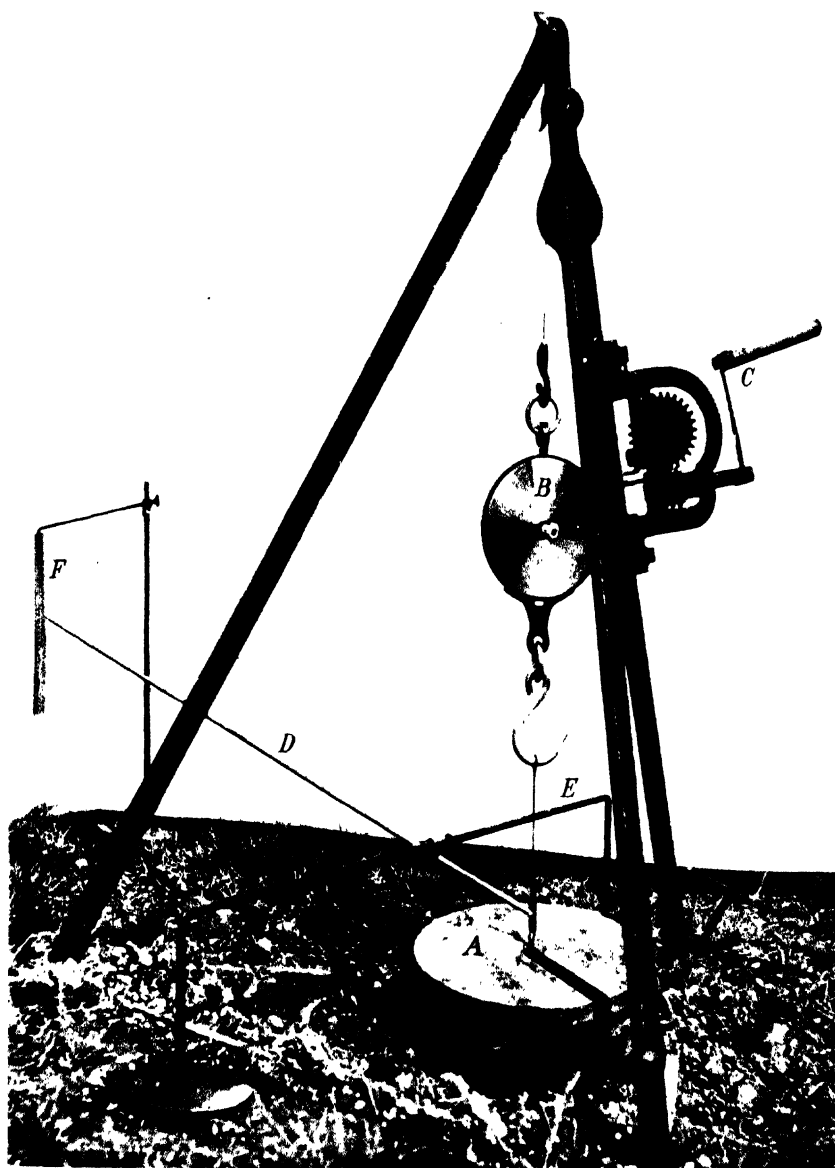
5. Preliminary field experiments are described in which the effect of simple cultivation processes on soil compressibility were measured.

6. Tentative conclusions about the significance of the differences in the shape of the laboratory curves are given, though these may need to be modified, and will certainly be extended following further experimentation.

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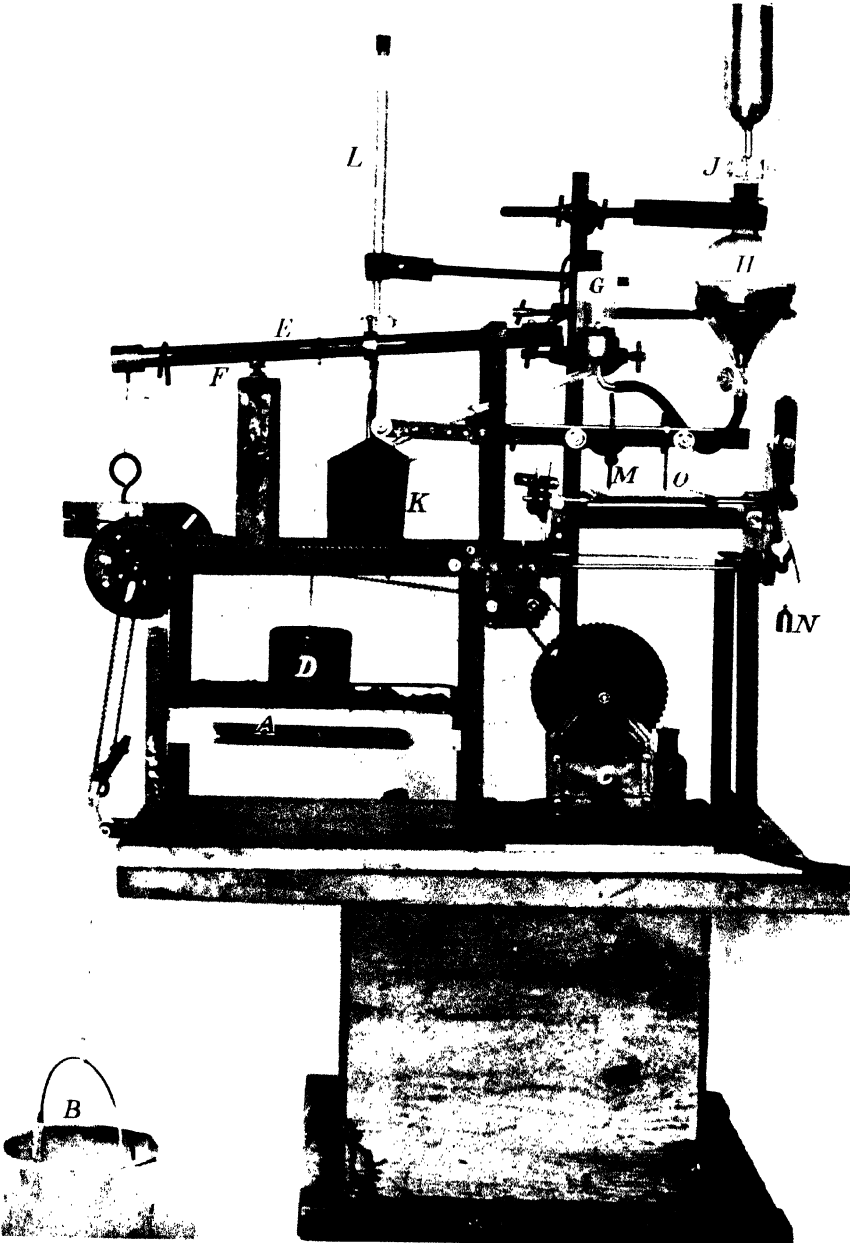
(Received 22 April 1937)



Field apparatus







Laboratory apparatus.



# BASE EXCHANGE EQUILIBRIA IN SOIL PROFILES

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THE exchange equilibria established between soil solution and adsorbed cations, determining the transfer of the exchangeable cations from one soil horizon to another, must be governed by the fundamental laws of base exchange. These have been investigated most completely by Wiegner and his co-workers<sup>(1-6)</sup>. Whilst the exchange properties of individual cations are relatively easily determined from simple experiments with clays and permutits, the equilibria associated with the processes of cationic migration in a soil profile are much more difficult to disentangle. Percolating waters containing basic cations and hydrogen ions in varying proportions come into contact with clay complexes produced by weathering processes, whose cation contents are not in equilibrium with those in the solution. Varying surface conditions may alter the ease of displacement of the adsorbed cations, and a similar effect may be produced in the solution by the presence of incompletely ionized salts.

## FACTORS AFFECTING CATION MIGRATION IN SOILS

The principal factors which must be considered are:

(1) The properties of the cations which are initially responsible for exchange reactions taking place. These are, in general, in moist temperate climates, the hydrogen ions produced in the upper horizons during the humification of organic residues. These, being potent displacing agents, may bring about direct exchange, or, by inaugurating further mineral weathering, set free other cations which take part in base exchange reactions. Carbonates or soluble salts may also supply exchange active cations to the percolating waters.

(2) The nature of the adsorbed cations and their relative amounts in the exchange complex. These are generally derived from the minerals of the parent material and are thus dependent on its geological origin. These adsorbed cations, after displacement by hydrogen in the upper horizons, become the exchange-active cations affecting the exchange complexes in the lower layers.

(3) The nature of the exchange complex: it has not been demonstrated

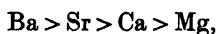
that results obtained for mineral colloids apply to the organic exchange material which abounds in the upper layers of most profiles. It is probable also that exchange in mineral colloids takes place both at clay-crystal-lattice and gel surfaces, and that these possess different adsorptive forces.

(4) The position on the surface of the complex at which the exchange is taking place: the influence of meta-structure on exchange equilibrium.

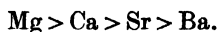
The general principles of base exchange on which any analysis of cation movement, in the light of the above factors, must be based, are due almost entirely to the Zürich school. First of all must be considered the order of ease of entry and displacement in the individual cationic series. For the univalent cations the order of ease of entry is:



whilst the most easily introduced ion is the most difficult to replace. This has recently been shown to apply also in the alkaline earth series, where the order of ease of entry is:



and of displacement:



Results obtained by the author for these cations have been quoted by Wiegner<sup>(5)</sup> and show that magnesium is more easily displaced than calcium, both from clays and permutits, whilst the exchange properties of calcium and strontium are practically identical.

These series appear to be best explained by the degree of hydration of the respective cations, the larger ions in any series being less hydrated and so able to approach more closely to the negative micellar charge, producing a complex less easily disturbed by external cations (and more easily coagulated). The effect of valency, doubling the cationic charge in the alkaline earths, renders comparison between the series difficult. The anomalous position of hydrogen is probably explained by the polarizing effect of the hydrogen ions on the water dipoles of the hydration sheath. Bär & Tendeloo<sup>(7)</sup> have attributed the lyotropic series directly to the polarization of the cations, but Giesecking & Jenny<sup>(8)</sup> have pointed out that the polarization effect alone is not sufficient to reverse the order which should be obtained on the basis of the ionic radii. The hydration theory appears also to provide the best explanation of "hindrance" effects noticed with small but hydrated cations in narrow capillaries. Thus, for example, the author has found that, with some permutits, magnesium to the extent of the total exchange capacity can only be introduced when the magnesium ions are partially dehydrated by using a

concentrated magnesium chloride solution. Only then, it appears, can the magnesium ions penetrate into the inner capillaries. Once introduced in this manner, the last traces of magnesium are difficult to displace: at high percentage displacements, the amount of calcium displaced may equal or exceed that of magnesium for the same concentration of displacing cation. It is possible that this effect is the cause of some of the statements that exchangeable magnesium is more firmly held in the soil than calcium—the magnesium remaining being held in capillaries whose entrances are too small for the fully hydrated magnesium ion.

Comparison of the properties of a uni- and a divalent cation which have similar exchange powers in high concentrations, e.g. calcium and sodium (1) shows that, in lower concentrations, the divalent cation enters the complex more readily. This is shown in the shapes of the exchange curves. The influence of the ion being replaced on the relative powers of the replacing cations is seen in some experiments of Renold (6) where it is shown that the more firmly bound the original cation, the less difference there is between the amounts displaced by cations of varying exchange powers.

Experiments with mixed Ca/Na solutions (all *N*/10 to chloride) on a Na-clay from the Sudan (13) gave the following results:

Ca/Na in solution	2.33	1.50	1.00	0.80	0.42	0.20	0.11	0.058
Ca/Na in clay	14.2	10.6	9.3	6.8	4.9	2.66	1.59	0.76

There is relatively 7–13 times as much Ca per unit Na in the equilibrium clay as in the solution, despite the fact that the clay was originally Na-saturated. This illustrates strikingly the difficulty of introducing univalent cations into an exchange complex in the presence of divalent cations, and moreover shows that one must expect little adsorption of such cations in the lower layers of a soil profile where Ca is also present in the percolating waters.

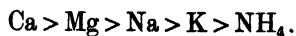
The effect of the surface position, or meta-structure, has been demonstrated in investigations of mixed  $\text{CaNH}_4$ -permutits, bentonites and clays made by Renold and the author (8,5), where it is shown that a cation occupying what is apparently an inner position in the pores or capillaries is more difficult to replace than one on the outer surfaces. This difference is least for the definitely structured bentonite and greatest for the very heterogeneous permutit, with kaolinite clay occupying an intermediate position. Calculations by Bär & Tendeloo (7) indicate that most of the exchange active surface of a soil clay must be capillary in nature, hence this effect is to be looked for also in soils. An attempt to produce a mixed  $\text{CaNH}_4$ -permutit from a mixed  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$  solution and a Na-permutit

gave a body with a higher  $\text{Ca}/\text{NH}_4$  ratio and in which the Ca occupied the inner positions, emphasizing the greater ease of entry and "penetration" of the divalent cation.

Consideration of the many base exchange results of Jenny and others emphasizes the importance of the history of a soil complex, as well as its present base status. These results show on recalculation that very differently saturated complexes may be in equilibrium with solutions of the same composition, and, conversely, that complexes containing the same cations in the same proportions may be in equilibrium with solutions of different compositions. The history of the complex, i.e. the position of the various cations on the surface, is the governing factor.

Exchange reactions in the mineral colloids of the soil may be of two kinds: double layer exchange at a gel-like surface, with hydroxyl ions forming the inner layer and the adsorbed cations the outer, and crystal lattice adsorption at clay mineral surfaces. The exchange properties of the latter probably vary according to the lattice structure. Bär & Tengel point out that the properties of adsorbed hydrogen must vary for the two types: that at a gel surface with an  $\text{OH}'$  inner layer tending to resemble adsorbed water. They consider also that the total base exchange capacity depends on the  $\text{pH}$  of the medium, as the number of  $\text{OH}'$  ions in the inner layer will vary with this value. The slight increase in the base exchange capacity of soils on liming, noted by numerous investigators, appears to be of the same magnitude as this effect. It is possible that anions other than  $\text{OH}'$  may constitute the inner layer, but at present the nature of anionic adsorption (of  $\text{PO}_4'''$ , etc.) is not clear. Such anions, if present, might account for the presence of truly exchangeable hydrogen at gel surfaces.

Most investigations of base movements in soil profiles up to the present have been confined to lysimeter and drainage water studies of the ions removed, and these have been summarized by Scharrer (12). The relative amount of the various cations removed in drainage is generally given by



This order is not, as is often stated, the relative ease of removal, as the amounts present in the soil must be considered, and, were this done, the order obtained would be



It is only because in normal humid soils calcium is responsible for some 80 per cent of the exchangeable base content that so much Ca is found in the drainage waters—it having been displaced by the hydrogen ions,

which, of the common soil cations, are alone able to remove it from the adsorption complex to any extent.

Most records of the exchangeable cation contents of soil profiles are incomplete; generally only Ca and Mg are recorded, with occasionally Na and K or H. The absence of any indication of the amount of exchangeable hydrogen present renders it practically impossible, especially with soils from the podzol zone, to study the relative movements of the cations down the profile, since, as pointed out above, the hydrogen is the chief displacing cation.

#### EXPERIMENTAL METHODS AND RESULTS

The Lundegårdh method of flame spectrographic analysis has rendered possible a more complete investigation of the exchangeable base content of soils. The results reported here were obtained by leaching with neutral normal ammonium acetate with a spectrographic analysis of the extract made as previously described (9). Exchangeable hydrogen and exchange capacity were determined by Parker's method (11) involving neutral normal barium acetate leaching for the exchangeable hydrogen and subsequent ammonium saturation, followed by distillation, to determine exchange capacity (given in brackets in the tables below).

Results are given for the exchangeable Ca, Sr, Mg, K, Na, Mn and H in six profiles, five from north-east Scotland and one from the south. The profiles selected, typical of some forty of the same type for which results are available, are of fairly high base exchange capacity in all layers, and are not so unsaturated that the hydrogen overshadows the other cations, as generally occurs in podzol profiles. The relationship of the high base exchange capacity of some layers to other soil characteristics (clay content and composition, drainage conditions, etc.) is at present being investigated (10), but in this communication it is proposed to deal solely with the base status of the various horizons and to point out in how far the present conditions are in accordance with theoretical considerations.

Most of the profiles so far investigated are developed on basic igneous till in north-east Scotland, but other profiles on quite distinct parent materials in other parts of Scotland show similar characteristics. Brief morphological descriptions and the experimental results for the various profiles follow.

**BB 40.** Brown soil on summit of Dunbennan Hill, Aberdeenshire, on till overlying troctolite.  
*Pinus sylvestris*, *P. montana*, *Larix decidua*, *Oxalis acetosella*, mosses, etc.

0-5 cm. Litter and root mat.

5-20 cm. Medium brown sandy loam. Crumbly with worm and root channels. Many stones. Damp.



20–60 cm. Sandy loam, slightly lighter in colour. Ochre staining in places. Worm and root channels. Slightly more compact.

60 cm. + Duller brown gritty loam with yellowish spots. Stones chiefly troctolite and picrite.

BB 40. *Exchangeable cations* (in m.e. per 100 g. <2 mm. soil):

Depth cm.	Ca	Sr	Mg	K	Na	Mn	H	Exchange capacity	Clay %	pH
5–20	2.6	0.013	6.2	0.19	0.75	0.164	13.9	25.3	—	22.9 5.85
20–30	2.2	0.015	7.0	0.24	0.71	0.017	9.6	19.8	—	18.8 5.11
50–60	4.1	0.024	23.0	0.52	0.53	0.004	5.4	33.6	(35.9)	17.2 6.77
90–100	4.9	0.045	27.0	0.54	0.62	0.008	—	32.9	(35.5)	16.0 7.15

BB 40. *Exchangeable cations* (as ratios of calcium content):

Depth cm.	Ca/Sr	Ca/Mg	Ca/K	Ca/Na	Ca/Mn
5–20	190	0.42	13.3	3.4	210
20–30	150	0.31	9.2	3.1	130
50–60	170	0.18	8.1	7.9	1100
95–100	110	0.18	9.1	8.1	630

BB 72. Brown soil on the west slope (5°) of the Bin, Aberdeenshire. Mixed basic igneous till overlying troctolite. Sitka spruce (planted), *Calluna* in clumps, *Deschampsia flexuosa*, *Nardus stricta*, *Viola*, *Potentilla*, *Anemone*, mosses.

0–25 cm. Medium brown loam. Crumbly. Yellowish brown spots towards bottom. Worm and root channels. Stones and boulders frequent.

25–45 cm. Yellowish brown loam with darker humus spots fairly compact. Stones (mixed). Roots ceasing mostly at 45 cm.

45–80 cm. Yellowish brown loam with fawn patches. Less compact. Stony.

80 cm. + Boulders or solid troctolite.

BB 72. *Exchangeable cations* (in m.e. per 100 g. <2 mm. soil):

Depth cm.	Ca	Sr	Mg	K	Na	Mn	H	Exchange capacity	Clay %	pH
0–10	6.3	0.009	2.1	0.35	0.26	0.025	18.8	27.8	—	20.2 5.87
15–25	4.3	0.012	2.3	0.15	0.37	0.008	6.5	13.5	—	17.0 5.93
25–35	3.8	0.011	2.0	0.05	0.30	0.005	6.9	13.0	(16.8)	12.5 6.00
70–80	10.9	0.034	25.2	0.10	0.83	0.004	4.9	43.0	(42.5)	11.6 6.53

BB 72. *Exchangeable cations* (as ratios of calcium content):

Depth cm.	Ca/Sr	Ca/Mg	Ca/K	Ca/Na	Ca/Mn
0–10	680	2.9	13.3	3.4	210
15–25	350	1.9	9.2	3.1	130
25–35	340	1.9	8.1	7.9	1100
70–80	320	0.4	9.1	8.1	630

BB 56. Gleyed soil on the middle slopes (3°) of Whitehill, near Rothiemay, Aberdeenshire. Mixed basic igneous till overlying hornblende schist. Vegetation consists of rushes, mosses and grasses.

0–20 cm. Dark brownish grey crumbly loam. Moist, with root and worm channels. many roots.

20–50 cm. Greenish fawn loam with slight rusty staining in spots. Roots down to about 50 cm. Becoming more compact.

50 cm. + Fawny brown loam with grey and rusty mottling which becomes more intense with depth. Water oozing in at 125 cm.

**BB 56.** *Exchangeable cations* (in m.e. per 100 g. <2 mm. soil):

Depth cm.	Ca	Sr	Mg	K	Na	Mn	H	Exchange capacity	Clay %	pH
0-10	3.1	0.008	0.35	0.54	0.18	0.010	17.9	22.1 (22.0)	14.6	5.46
20-30	1.1	0.003	0.38	0.13	0.27	0.003	4.5	6.3 (7.6)	9.5	5.76
50-60	4.9	0.012	2.6	0.12	0.45	0.016	0.9	8.0 (8.7)	11.3	6.96
120-130	6.1	0.010	3.0	0.14	0.22	0.023	0.4	9.9 (10.2)	13.1	7.10

**BB 56.** *Exchangeable cations* (as ratios of calcium content):

Depth cm.	Ca/Sr	Ca/Mg	Ca/K	Ca/Na	Ca/Mn
0-10	400	8.9	5.8	17.0	330
20-30	330	2.8	8.1	3.9	350
50-60	400	1.9	40.0	10.8	310
120-130	610	2.0	44.0	28.0	270

**BB 78.** Gleyed soil on west side of Hill of Mungo, Aberdeenshire. Till overlying norite. *Nardus*, *Agrostis*, *Viola*, *Potentilla*, *Galium saxatile*, *Hylocomium loreum* and *Brachythecium purum*.

- 0-12 cm. Brownish grey humose loam. Many roots, occasional stones.  
 12-21 cm. Similar colour, less humus. Granular structure. Roots and worm channels.  
 21-31 cm. Greyish brown with rusty mottling, fairly compact loam. Stones—fewer roots.  
 31-130 cm. Mottled bluish grey, rusty, and medium brown. Compact loam. Very few roots. Stones mixed, basic igneous, quartzite and slates.

**BB 78.** *Exchangeable cations* (in m.e. per 100 g. <2 mm. soil):

Depth cm.	Ca	Sr	Mg	K	Na	Mn	H	Exchange capacity	Clay %	pH
0-12	5.3	0.012	2.0	0.30	0.50	0.055	9.3	17.5 (21.0)	22.4	5.44
12-21	3.4	0.007	1.1	0.22	0.26	0.011	9.5	14.5 (16.9)	20.1	5.55
21-29	1.7	0.003	0.8	0.13	0.20	0.005	6.0	8.4 (8.5)	13.3	5.85
31-41	3.5	0.009	1.8	0.11	0.27	0.017	2.4	8.2 (10.0)	16.2	5.91
70-80	7.5	0.010	4.0	0.05	0.36	0.017	1.6	13.5 (11.9)	15.7	6.57
120-130	7.2	0.012	4.8	0.08	0.48	0.020	1.6	14.2 (12.6)	17.5	6.49

**BB 78.** *Exchangeable cations* (as ratios of calcium content):

Depth cm.	Ca/Sr	Ca/Mg	Ca/K	Ca/Na	Ca/Mn
0-12	430	2.6	18	11	96
12-21	510	3.1	15	13	300
21-29	550	2.2	13	9	340
31-41	380	1.9	31	13	210
70-80	740	1.9	180	21	440
120-130	590	1.3	74	13	250

**BB 75.** Slightly podzolized soil with slight gleying, at Clean Pool, Ordiquhill, Aberdeenshire. Till overlying contaminated basic igneous rocks. Scots pine, *Calluna*, *Vaccinium*, grasses and mosses.

- 0-5 cm. Brown *Calluna* mor.  
 5-6 cm. Dark grey layer, lightens on drying.  
 6-20 cm. Brown loam with greyish spots.  
 20-40 cm. Mottled yellowish brown loam.  
 40 cm. + Light loam with slight rusty and grey mottling.

BB 75. *Exchangeable cations* (in m.e. per 100 g. <2 mm. soil):

Depth cm.	Ca	Sr	Mg	K	Na	Mn	H	Exchange capacity		Clay %	pH
0-5	12.6	0.040	6.6	1.9	1.0	—	64.8	87.0	—	—	4.55
5-6	2.4	0.009	1.5	0.43	0.37	—	30.0	34.7	—	12.9	4.89
6-16	0.19	0.0005	0.90	0.16	0.29	—	41.2	42.7	(37.9)	24.5	4.83
20-30	0.17	0.0006	0.18	0.14	0.19	0.005	33.4	34.1	(34.2)	25.4	5.04
40-50	2.5	0.006	1.1	0.20	0.31	—	7.1	11.2	(11.7)	15.3	5.74
100-110	7.7	0.013	3.4	0.13	0.58	0.019	1.3	13.2	—	9.1	6.14

BB 75. *Exchangeable cations* (as ratios of calcium content):

Depth cm.	Ca/Sr	Ca/Mg	Ca/K	Ca/Na	Ca/Mn
0-5	320	1.9	6.8	12.5	—
5-6	270	1.7	5.8	6.6	—
6-16	420	0.2	1.2	0.7	—
20-30	300	0.9	1.2	0.9	34
40-50	430	2.2	13.0	8.3	—
100-110	590	2.3	57.0	13.1	400

WM 4. Peat gley soil. Wull Muir, Moorfoot Hills, Midlothian. Silurian shale. *Eriophorum vaginatum*, *Carex Goodenowii*, *Deschampsia flexuosa* and *Festuca ovina*. Spots of *Calluna*.

0-4 cm.	Root mat.
4-30 cm.	Fibrous peaty material, roots abundant.
30-37 cm.	Greyish silty loam with organic staining. Decaying roots.
37-50 cm.	Grey fine sandy loam, damp, many roots, green patches, brown staining.
50-65 cm.	Greenish grey silt, gritty and slightly stony. Damp.
65-95 cm.	Greyish brown gritty and stony silty loam, many greyish green patches.
95 cm. +	Brown gritty and stony loam. Clayey, wet, stony-shattered shale, giving greyish green patches.

WM 4. *Exchangeable cations* (as m.e. per 100 g. <2 mm. soil and as ratios):

Depth cm.	Ca	Sr	Mg	H	pH	Ca/Sr	Ca/Mg
0-4	5.5	0.016	—	27.8	4.3	350	—
4-30	7.3	0.023	2.9	49.1	3.9	310	2.5
30-37	2.1	0.005	1.3	12.3	4.7	400	1.6
37-50	0.9	0.0014	0.8	1.2	5.1	640	1.1
50-65	4.0	0.009	2.1	2.3	5.0	420	1.9
65-95	9.2	0.014	4.1	0.6	6.0	680	2.2
150-165	13.3	0.025	4.6	0.3	6.7	540	2.9

## DISCUSSION AND CONCLUSIONS

The exchange capacity in general shows no marked irregularities throughout the profiles. In most cases it follows the clay content (<0.002 mm.) fairly closely, with an exchange value of approximately 1 m.e. per g. of clay. Profiles BB 72 and BB 40 have exceptionally high exchange capacities in the lower layers, associated with high exchangeable magnesium contents. The magnesium is truly exchangeable, as it can be replaced by ammonium and the ammonium subsequently recovered, as

the exchange capacity figures by the Parker method show. The presence of organic matter increases the exchange capacities in the surface horizons.

Exchangeable hydrogen decreases steadily down the profiles, being highest in the upper horizons, where acid conditions develop during the humification of organic residues. These upper horizons are, however, not completely unsaturated, as the litter supplies an appreciable quantity of other cations, which keep the  $pH$  above 5, except in WM 4 and BB 75, both of which show definite evidence of podzolization. The decrease in exchangeable hydrogen content with depth is reflected in the rise in  $pH$ , the bottom layers approaching neutrality.

Little can be gained by a study of the individual cation contents of the organic layers, where the displaced bases are replaced by the supply from the litter. These have been obtained by the vegetation either from the percolating waters or directly from the exchange complex, presumably completing the equilibrium by supplying hydrogen to the complex. Thus the bases in the surface horizons will depend to a great extent on the amounts taken up by the roots, and this tends to upset base exchange equilibria.

The percolating solutions from the upper horizons are somewhat acid and, as a result, in the middle layers there has been a displacement of the original cations of the exchange complex. Thus it is seen that, in these horizons, the calcium content in particular falls to its minimum value.

Considering first of all the divalent cations, there is a minimum content of these bases at about 30 cm. depth, and below this the amounts rise considerably. At this point the displacement of other cations by hydrogen has reached its maximum and calcium becomes the chief cation in the percolating waters. Any exchange occurring lower down the profile is the displacement of univalent cations by this calcium. Thus in the lower layers there is a high content of calcium and the other divalent cations, due to their initial preponderance and to the slight accumulation of calcium from the middle layers at the expense of the alkalis. The ratios of calcium to strontium and of calcium to magnesium are interesting, the former especially so. There is, in all the soils except BB 40, about 400 times as much exchangeable calcium as exchangeable strontium, and this ratio is reasonably constant in all the layers of the profiles, both where displacement by hydrogen and subsequent slight accumulation have occurred. Thus it seems that the similarity in the exchange properties of calcium and strontium, previously noted for the cations individually, holds also where these cations are present in vastly different proportions and in the

presence of other cations. There is no preferential movement of the large quantities of calcium or retention of the last traces of strontium. This points to the calcium and strontium being intimately mixed on the exchange surface and neither favouring the outer surfaces or pores. This is, of course, what would be expected from their properties and the fact that they were simultaneously introduced into the complex during its formation from the products of weathering. As far as movements of calcium and magnesium are concerned, the Ca/Mg ratio is in general higher in the layers which have the lowest base contents than in the unleached bottom layers, pointing to the easier removal of magnesium. This may be complicated by the quicker downward movement of magnesium from the surface litter, as seems to be the case in BB 75. On the other hand, the relatively high amounts of magnesium in the leached layers may be due to the anomalous behaviour of magnesium previously mentioned. The high magnesium content of some of the parent materials, e.g. of BB 40 and BB 72, which contain picrites, also confuses the issue, but as the magnesium contents of the upper layers have fallen to normal, this also points to the easier removal of magnesium. There appears to be a drop in the base exchange capacity when the soil material undergoes further weathering in the upper layers. This, however, is at present being investigated.

Manganese has not been studied quantitatively to any extent and the results obtained are too irregular to allow any definite conclusions to be drawn. The state of oxidation (gleying, water table, etc.) must, however, influence the amount and movement of manganese considerably.

Sodium and potassium do not show the marked increase in the lower horizons seen with the divalent cations. Sodium in some instances does show a slight increase, displacement by calcium being slower than by hydrogen, but potassium gives no evidence of an accumulation. This may be because much of the potassium does not pass down in the drainage water but is taken up by plants and accumulates in the litter. While the volume content of potassium in the organic layers may be little, if any, higher than that in the mineral layers, it must be remembered that the organic layers and the vegetation are new formations and, therefore, all the potassium found in these layers and in the vegetation has been removed from lower layers. The potassium that does remain in the exchange complex is possibly in the inner pores and, therefore, more difficult to replace, and less available to the plants. The increasing Ca/Na ratios down the profile point to the greater exchange activity of Ca and are what would be expected from the results on p. 559. In the lower layers the

Ca/Na ratio of 10 in the complex suggests, from these results, a ratio of between 2 : 1 and 1 : 1 in the solutions. Analyses of drainage waters from soils of this type are unfortunately not available.

From a consideration of these results it appears that, while the data are not conclusive on all points, the movements of cations occurring in the profiles investigated follow the lines indicated by theoretical conditions. The most significant result is probably the similar rate of movement of calcium and strontium, cations with similar exchange properties, even when such a wide atomic ratio as 400 : 1 exists in the quantities present.

### SUMMARY

1. The conditions governing base exchange equilibria in soil profiles are discussed.
2. Exchangeable cation contents of six profiles are reported, the results including figures for Ca, Sr, Mg, Na, K, H and exchange capacity, as well as clay content and *pH* value.
3. It is shown that cation displacement occurs as a result of the downward movement of hydrogen ions from the surface layers, and a layer of minimal basic cation content exists at about 30 cm. depth.
4. Below this depth the basic cation content increases once again, but the base exchange capacity follows the clay content fairly closely.
5. Calcium and strontium are displaced (and readsorbed) at similar rates, in accordance with their exchange constants, although there is about 400 times as much calcium as strontium present. The behaviour of the other cations also appears to be in accordance with theoretical demands.

The author desires to acknowledge the help of Miss A. I. Bell, who has carried out much of the routine work associated with the investigation, and to thank Dr Alex Muir for putting the profiles and descriptions at his disposal.

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# THE NUTRITION OF THE BACON PIG

## II. THE INFLUENCE OF HIGH-PROTEIN INTAKE ON PROTEIN AND MINERAL METABOLISM

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### INTRODUCTION

INVESTIGATIONS into the protein requirements of pigs have usually in the past been carried out by determining the rate of live-weight increase of pigs fed as groups on rations of known protein content. The results of such investigations have led to the belief that, for purposes of a satisfactory rate of growth, a ration supplying about 10 per cent of a food such as white fish meal, containing a large percentage of protein of high biological efficiency, is adequate from the stage of weaning to 150 lb. live weight; whilst from 150 lb. to slaughter at about 200 lb. live weight, the supply of protein-rich food in the ration may safely be reduced to about 5 per cent.

Although these conclusions have received confirmation from the results of isolated metabolism trials, little systematic research appears to have been done on the utilization of the food nitrogen for the building up of body flesh. The question as to whether an increase of the protein supply beyond the customary levels results in a corresponding increase in the rate of deposition of lean flesh in the body of the animal has, in particular, received but scant attention.

In large-scale feeding trials carried out recently, the writers found that an unusually heavy replacement of cereal by protein-rich food in the diets of Large White bacon pigs had only the slightest effect on the rate of live-weight increase between weaning and slaughter (1). The diet containing an abnormally high percentage of protein-rich food gave rise to carcasses neither leaner nor fatter than those arising from a diet containing a normal amount. From the standpoints of both growth and fattening, therefore, the high-protein diet was scarcely to be distinguished from the normal-protein diet. It was found, further, that the gilts in the experiment produced somewhat leaner carcasses than hogs from the same litter, a finding that pointed to a somewhat more efficient retention of food nitrogen by the gilts.



Since the factors which determine the leanness of bacon carcasses are of considerable economic importance, it was decided to investigate further, by the more precise technique of metabolism trials, the utilization of food protein by bacon pigs at different stages of growth from weaning to slaughter. At the same time the opportunity was taken for securing information relating to the retention of lime, phosphoric acid and chlorine.

#### EXPERIMENTAL PROCEDURE

The Large White pigs used in this trial came from the litter of a sow which had farrowed on 9 August 1935. The litter was weaned when 8 weeks old, and four pigs (two gilts and two hogs) were chosen so as to form as uniform a group as possible. Their live weights at this stage ranged from 37½ to 41 lb. After arrival at the metabolism buildings, the piglets were "wormed" and then brought gradually on to the experimental diets.

The feeding treatments are shown in Table I and are referred to as the "normal-protein" and the "high-protein" diets. The normal-protein rations contained the amounts of white fish meal customarily used in pig-feeding, whilst the high-protein rations were derived from them by the addition of 12 per cent of ex. soya-bean meal in replacement of an equal weight of barley meal. The size of the ration was adjusted throughout the experiments to conform with the live weight of the pigs, the standards given in the publication already referred to being followed as closely as possible (1).

It will be noted that no mineral supplement was added to the rations when the amount of white fish meal was reduced to 5 per cent at 150 lb. live weight. This omission was deliberate, since it was desired to ascertain whether the use of a mineral supplement is actually necessary at this stage when white fish meal is being used at this rate.

Table I. *Summary of feeding treatments*

Range of live weight	Weaning to 90 lb.		90-150 lb.		150-200 lb.	
	Normal-protein diet	High-protein diet	Normal-protein diet	High-protein diet	Normal-protein diet	High-protein diet
Barley meal	55	43	65	53	75	63
Weatings	31	31	23	23	18	18
Lucerne meal	2	2	2	2	2	2
White fish meal	12	12	10	10	5	5
Ex. soya-bean meal	—	12	—	12	—	12

One gilt and one hog were kept on the normal-protein rations, whilst the other pair was kept throughout on the high-protein diet. The balance trials were of 14 days' duration, collection and analysis of the excreta being carried out during the last 10 days. The design of the metabolism cage has been described in detail in a previous publication, which also gives an account of the general conditions to be observed in the carrying out of such work (2). For the purpose of the present experiments, the movable dividing partition mentioned in this earlier description was fixed so as to enable two animals to be under experiment at the same time. With a view to facilitating the collection of the dung, each compartment was provided with a vertically sliding partition which could be lowered into position at collection times. By this means the pig could be confined to one-half of the compartment whilst the dung in the other half was being collected.

On the morning of 10 October 1935, the gilt on the high-protein diet and the hog on the normal-protein diet were placed in the cages for the first metabolism period. This lasted 14 days, when the pigs were removed from the cages and returned to the indoor pens in which they were kept during the intervals between experiments. Their places in the cages were then taken by the second pair of animals, the hog now being on the high-protein diet and the gilt on the normal-protein food. After completing this second trial, the first pair of pigs were again brought into experiment, and by following this procedure it was found possible to carry out seven separate metabolism experiments before the animals arrived at slaughter weight.

Table II. *Composition of feeding stuffs (dry-matter basis)*

Period	Barley meal		Weatings		White fish meal		soya-bean meal	Lucerne meal†
	1-5 %	6-7 %	1-5 %	6-7 %	1-5 %	6-7 %	1-7 %	1-7 %
Crude protein	13.54	14.61	17.82	18.54	70.98	70.58*	50.62	26.45
Ether extract	2.96	3.45	3.91	6.52	4.77	4.75	0.71	3.62
N-free extractives	74.68	72.75	69.79	66.22	—	—	35.19	40.20
Crude fibre	5.95	5.58	5.38	5.15	—	—	6.66	18.78
Ash	2.87	3.61	3.10	3.57	24.25	26.51	6.82	10.95
Lime (CaO)	0.169	0.103	0.160	0.138	10.421	9.714	0.441	3.821
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.611	0.838	1.473	1.583	8.801	9.359	1.492	0.701
Chlorine (Cl <sub>2</sub> )	0.127	0.137	0.090	0.068	1.367	1.895	0.044	0.939

\* The crude protein, ether extract and ash in this column add to 101.84 per cent, an anomaly due to the use of the conventional factor 6.25 for converting nitrogen into crude protein.

† Lucerne cut high, so as to give a large proportion of leaf.

Before each trial the rations for the whole period were weighed out into paper bags and the moisture content of the foods was determined. The composition of the feeding stuffs employed in the trials is shown in Table II.

#### THRIFTINESS OF PIGS ON EXPERIMENTAL RATIONS

No difficulty was experienced in securing consumption of the high-protein rations. The faeces of the pigs on this diet were firm and normal, the suggestion frequently made that high-protein feeding necessarily causes "scouring" receiving no support from this trial or from the large-scale feeding trial referred to previously (1). That the treatment of the pigs throughout the experiments had no disadvantageous influence on thriftiness is revealed by the figures in Tables III and IV.

Table III. *Live-weight gains of pigs during metabolism trials*

Age in days	Date	First pair		Second pair	
		High-protein diet Gilt 1410 Live weight lb.	Normal-protein diet Hog 1405 Live weight lb.	High-protein diet Hog 1404 Live weight lb.	Normal-protein diet Gilt 1406 Live weight lb.
56	4 Oct.	41	39	38	37½
62	10 Oct.	46	45½	42½	41
76	24 Oct.	54	51½	—	—
80	28 Oct.	—	—	61	61
94	11 Nov.	78½	78½	73	74½
108	25 Nov.	98½	97	94	98
122	9 Dec.	120	118	111	116
136	23 Dec.	147½	149	—	—
143	30 Dec.	156	154	—	—
157	13 Jan.	178	173	—	—
164	20 Jan.	187	185	175	177
173	29 Jan.	204	198	—	—
178	3 Feb.	—	—	203	201

Table IV. *Showing mean daily live-weight increases and food conversion factors from beginning of trial on 10 October to date of attainment of 200 lb. live weight*

No. of pig	Sex	Feeding treatment	No. of days	Total L.W.I. lb.	Mean daily L.W.I. lb.	Total meal consumed lb.	Meal per lb. L.W.I. lb.
1410	Gilt	High-protein	110	154	1.40	486.5	3.16
1405	Hog	Normal-protein	112	155	1.38	500.6	3.23
1404	Hog	High-protein	115	157½	1.37	531.3	3.37
1406	Gilt	Normal-protein	116	159	1.37	521.4	3.28

The results in Tables III and IV show that the four pigs under experiment displayed a high degree of thriftiness, the ages at which they attained 200 lb. live weight varying from 6 months 4 days in the case of

gilt 1410 to 6 months 10 days for gilt 1406. Extremely satisfactory live-weight gains were registered over the periods when the animals were in the metabolism cages, and the average figures for daily live-weight increase and efficiency of food conversion over the entire experimental period compare well with the corresponding figures for pigs fed in an ordinary piggery under very good conditions of management.

It is clear, therefore, that the experimental treatment to which the pigs were subjected led to no retardation of the rate of growth, and that the findings of the present work can legitimately be applied to pigs being fed with a view to securing the quick rate of growth required by modern standards of bacon production.

It will also be noted that there was little or nothing to distinguish the rates of growth and the efficiencies of food conversion for the pigs on the normal-protein and the high-protein diets, a finding in harmony with the conclusions from the earlier pig-feeding trial reported in the first of this series of publications (1).

#### DIGESTION RESULTS

In order to ascertain whether the extra protein in the high-protein rations exerted any marked influence on the extent to which the food was digested, it was considered desirable to determine the digestion coefficients of the dry matter, organic matter and protein in the rations fed during each metabolism experiment. The results of these determinations are shown in Table V.

Table V. *Summary of digestion results*

Diet	No. of pig	Period*	Digestion coefficients		
			Dry matter %	Organic matter %	Crude protein %
Normal-protein	Hog 1405]	1	78.50	80.30	82.13
High-protein	Gilt 1410]		79.81	81.36	83.10
Normal-protein	Gilt 1406]	2	78.82	81.00	82.18
High-protein	Hog 1404]		78.90	81.28	83.12
Normal-protein	Hog 1405]	3	79.43	81.78	81.81
High-protein	Gilt 1410]		78.02	80.26	82.39
Normal-protein	Gilt 1406]	4	78.50	80.62	81.48
High-protein	Hog 1404]		77.97	80.48	81.93
Normal-protein	Hog 1405]	5	80.56	82.75	83.61
High-protein	Gilt 1410]		79.64	81.77	82.33
Normal-protein	Hog 1405]	6	78.68	80.90	81.60
High-protein	Gilt 1410]		79.70	81.46	83.06
Normal-protein	Gilt 1406]	7	79.14	80.99	81.04
High-protein	Hog 1404]		79.51	81.46	83.20

\* Periods 1, 2 and 3: diets as fed from weaning to 90 lb. live weight; periods 4 and 5: diets as fed from 90 to 150 lb. live weight; periods 6 and 7: diets as fed from 150 to 200 lb. live weight.

The digestion coefficients in Table V show that the animals suffered from no digestive disturbances during the course of the metabolism trials. It is of interest to note that the young pigs after weaning were able to digest their food with as high an efficiency as was displayed in the later stages of growth. The results show further that the extra protein in the high-protein rations had little or no effect on the extent to which the food was digested.

#### PROTEIN METABOLISM

In addition to the conventional analytical determinations involved in nitrogen balance experiments, it was thought desirable, in order to secure a more complete picture of the protein metabolism in the pigs, to make estimations of the different nitrogenous compounds in the composite samples of urine. Sulpho-salicylic acid tests were carried out as well as boiling tests for coagulable protein. Apart from an occasional very slight turbidity, no evidence was secured at any stage of the trials suggesting the presence of protein in the urine of the pigs subsisting on either the normal-protein or the high-protein diets. Determinations of urea and ammonia were made on the composite samples of urine from each metabolism experiment. The results of the nitrogen metabolism studies are shown in Table VI.

One of the most striking conclusions to be drawn from the results in Table VI is that the gilts showed consistently a higher rate of protein storage than the hogs. This behaviour was manifested even when the hog was receiving the high-protein diet, and the gilt, with which the hog was being compared, was receiving a lower supply of protein in the form of the normal-protein diet. This more efficient utilization of food protein by the gilts is in harmony with the finding from the earlier large-scale feeding trial (1) that the gilts gave somewhat leaner carcasses than the hogs.

An analysis of the nitrogen balances in Table VI shows that nitrogen retention from the high-protein diet was no higher than from the normal-protein diet. During periods 2-7, in which gilts and hogs are represented equally in relation to the two feeding treatments, the total nitrogen retained by the normal-protein pigs over the 60 days on which the nitrogen balances were determined amounted to 841 g. The amount retained by the high-protein pigs over the same 60 days was almost equal, namely, 845 g., although these animals had consumed 874 g. nitrogen (i.e. 5463 g. protein) in excess of the intake of the normal-protein pigs.

It is clear, therefore, that the extra protein in the high-protein rations was not utilized for the production of lean muscle in the body. It must be

Table VI. *Summary of results in nitrogen metabolism studies*

Period	No. of pig and sex	Mean L.W. in period lb.	Diet*	Total N con- sumed per day g.	N voided per day			Mean daily N balance g.	N excreted per day as			Extra protein deaminated by H.-P. pig per day g.	L.W.I per 14 days lb.
					Faeces g.	Urine g.	Total g.		Urea g.	NH <sub>3</sub> g.	Creati- nine, etc. g.		
1	(1405 H.	48	N.P.	28.09	5.02	11.12	16.14	11.95	8.04	0.86	2.22		6
	(1410 G.	50	H.P.	33.85	5.72	14.47	20.19	13.66	10.71	1.12	2.64	16.7	8
2	(1406 G.	67.8	N.P.	43.59	7.77	22.92	30.69	12.90	15.85	3.12	3.95		13½
	(1404 H.	67	H.P.	52.61	8.88	32.21	41.09	11.52	26.73	1.35	4.13	68.0	12
3	(1405 H.	87.8	N.P.	59.12	10.75	33.99	44.74	14.38	25.72	3.26	5.01		18½
	(1410 G.	88.5	H.P.	71.22	12.54	42.57	55.11	16.11	34.18	2.57	5.82	52.9	20
4	(1406 G.	107	N.P.	61.06	11.31	35.22	46.53	14.53	25.27	2.96	6.99		18
	(1404 H.	102.5	H.P.	74.50	13.46	51.41	64.87	9.63	41.88	2.48	7.05	103.8	17
5	(1405 H.	133.5	N.P.	73.08	11.98	46.60	58.58	14.50	34.82	5.04	6.74		31
	(1410 G.	133.8	H.P.	89.00	15.73	56.66	72.39	16.61	45.32	3.31	8.03	65.6	27½
6	(1405 H.	163.5	N.P.	71.65	13.18	45.53	58.71	12.94	31.41	4.44	9.68		19
	(1410 G.	167	H.P.	88.71	15.03	57.10	72.13	16.58	44.94	3.14	9.02	84.6	22
7	(1406 G.	189	N.P.	81.82	15.51	51.45	66.96	14.86	37.51	6.15	7.79		24
	(1404 H.	189	H.P.	101.70	17.09	70.53	87.62	14.08	54.40	5.45	10.68	105.6	28

\* N.P. = normal-protein; H.P. = high-protein.

Periods 1, 2 and 3: diets as fed from weaning to 90 lb. live weight; periods 4 and 5: diets as fed from 90 to 150 lb. live weight; periods 6 and 7: diets as fed from 150 to 200 lb. live weight.

concluded that the protein supply in the normal-protein diet was sufficient to meet the demands for quick growth in the pigs, and that the extra protein in the high-protein diet, instead of being used for increasing the rate of building up new body protein, underwent deamination, a transformation which accounts for the much higher elimination of urea in the urine of the pigs receiving the high-protein rations. If the output of urea be taken as an index of the extent of deamination, it is of interest to note, from the figures in Table VI, that of the 874 g. of extra food nitrogen consumed by the high-protein pigs over the 60 days constituting periods 2-7, as much as 769 g. is accounted for by the nitrogen of the extra urea eliminated in the urine of these pigs.

The foregoing findings, therefore, afford a scientific basis for explaining the main result of the earlier pig-feeding trial, namely, that increasing the protein intake of bacon pigs to levels far beyond those used in ordinary feeding practice led to no gain whatsoever in respect of carcass leanness (1).

There is no indication from the figures in Table VI that the rate of deposit of body protein in the bacon pig increases with increasing live weight. Indeed, a general survey of the nitrogen balances points to the surprising conclusion that the daily nitrogen retention remains very much the same throughout the whole period from weaning to 200 lb. live weight. Even when the pigs were increasing in weight at the rate of about 2 lb. per day (period 5), the nitrogen retention was of the same order as in the other periods when the rate of gain was much smaller.

If it be assumed that the rate of retention of nitrogen is roughly constant from weaning to 200 lb. live weight, it follows, since the rate of live-weight increase rises continuously from  $\frac{1}{2}$ -1 lb. per day in the early stages to  $1\frac{1}{2}$ -2 lb. in the later stages, that the deposition of lean becomes an increasingly smaller proportion, and of fat an increasingly larger proportion, of the total deposit in the body as growth and fattening proceed. Since the formation of body fat required more starch equivalent than the formation of an equal weight of body protein, and since in addition the moisture content of the live-weight increase is decreasing continuously throughout growth, it is not difficult to understand why the amount of meal required per lb. of live-weight gain undergoes a steady and continuous increase from weaning to slaughter weight.

It should be noted that the maintenance of a fair degree of constancy in the daily nitrogen balance throughout growth and fattening was manifested despite the reduction of the amount of white fish meal from 12 per cent in periods 1, 2 and 3 to 10 per cent in periods 4 and 5 and to 5 per cent in periods 6 and 7. It should be kept in mind, however, that

the other foods in the normal-protein ration (barley meal, lucerne meal and weatings) also functioned as sources of protein, and that the reduction of the amount of white fish meal was more than counterbalanced from the protein standpoint by the steadily increasing weight of meal consumed by the pigs as they grew older.

It is of interest at this stage to consider the relation of the foregoing findings to certain measurements that were made on the carcasses following slaughter of the pigs. The technique of this post-slaughter work has been explained fully in a previous paper (1). It is merely necessary to explain that the term "complete rasher" refers to the surface exposed in the side of cured bacon by cutting at a point in the back between the fourth and fifth ribs (counting from the gammon end) right through to the belly. The figures are given in Table VII, together with the mean daily nitrogen balances for the pigs in the metabolism trials.

Table VII. *Summary of post-slaughter results together with mean values for nitrogen retention*

No. and sex of pig	Diet	Mean thick- ness of back fat cm.	Mean thick- ness of belly streak cm.	Complete rasher						Mean daily N reten- tion in meta- bolism trials g.
				Total area sq. cm.	Area of lean sq. cm.	Area of fat sq. cm.	Area of bone sq. cm.	% of total area as		
								lean %	fat %	
1405 H.	N.P.	3.72	4.01	220.2	65.5	150.9	3.8	29.75	68.53	13.44
1410 G.	H.P.	3.57	3.27	209.0	74.6	127.9	6.5	35.69	61.19	15.74
1406 G.	N.P.	3.72	4.01	218.2	72.6	137.3	8.3	33.27	62.92	14.09
1404 H.	H.P.	4.57	4.18	246.1	64.0	176.9	5.2	26.00	71.88	11.74

The figures in Table VII are not given for the purpose of drawing definite conclusions about the influence of protein supply on the post-slaughter measurements, since this has already been done in the report on a previous feeding trial, in which the experimental lay-out was specially designed to enable such conclusions to be drawn with safety (1). That carcass leanness was not determined by the magnitude of the protein supply, however, is evident, since although the high-protein gilt 1410 had the thinnest back fat and the leanest rasher, this is off-set by the fact that the high-protein hog 1404 had the thickest back fat and the fattest rasher. Both gilts gave a leaner rasher than their brother hogs, a finding in harmony with the higher nitrogen retention consistently shown by the gilts in the metabolism experiments. Indeed, it is clear that the values for nitrogen retention would have enabled a correct prediction to have been made of the relative leanness of the carcasses, since there is a striking parallelism between the mean nitrogen balances and the figures expressive



of the leanness of the rasher. Such harmony lends additional weight to the significance to be attached to the results of the metabolism studies.

### MINERAL METABOLISM

*Lime balances.* A summary of the results of the lime balances in the different periods of the metabolism experiments is given in Table VIII.

Table VIII. *Summary of lime balances*

Period	No. of pig and sex	Mean L.W. during period lb.	Diet	CaO in daily ration g.	Mean CaO eliminated daily		Mean daily CaO balance g.	Mean daily N balance g.
					In urine g.	In faeces g.		
1	1405 H.	48	N.P.	12.01	0.15	5.68	6.18	11.95
	1410 G.	50	H.P.	12.27	0.22	5.50	6.55	13.66
2	1406 G.	67.8	N.P.	18.69	0.31	11.12	7.26	12.90
	1404 H.	67	H.P.	19.11	0.31	12.64	6.16	11.52
3	1405 H.	87.8	N.P.	25.24	0.19	15.41	9.64	14.38
	1410 G.	88.5	H.P.	25.80	0.47	15.48	9.85	16.11
4	1406 G.	107	N.P.	23.96	0.28	14.84	8.84	14.53
	1404 H.	102.5	H.P.	24.58	0.27	17.89	6.42	9.63
5	1405 H.	133.5	N.P.	28.65	0.36	17.19	11.10	14.50
	1410 G.	133.8	H.P.	29.38	0.42	18.52	10.44	16.61
6	1405 H.	163.5	N.P.	16.45	0.29	12.30	3.86	12.94
	1410 G.	167	H.P.	17.45	0.56	12.16	4.73	16.58
7	1406 G.	189	N.P.	18.83	0.62	13.08	5.13	14.86
	1404 H.	189	H.P.	19.99	0.59	14.42	4.98	14.08

It will be noted from Table VIII that the rate of lime retention showed no tendency to fall in the first five periods, at the end of which the pigs had reached about 150 lb. live weight. In periods 6 and 7 (150–200 lb. live weight), however, the lime balances fell to a distinctly lower level. It is difficult to believe that at this stage the actual lime requirements of the pigs underwent a sudden fall. The diminution in the lime balances is rather to be ascribed to the fall in the lime supply of the ration consequent on the reduction of the white fish meal from 10 to 5 per cent, and the figures suggest that it would have been advisable at this stage to have supplemented the ration with lime. In practice this supplement would be given in the form of 1 per cent of a mixture of 3 parts of ground chalk and 1 part of common salt, the amount being doubled when the white fish meal is replaced by an equal weight of mineral-deficient protein food such as ex. soya-bean meal.

If the lime balances be compared with the corresponding phosphoric acid balances in Table IX, it will be seen that lime retention exceeded phosphate retention so long as 10 per cent of white fish meal was being fed, but that the reverse held true following the reduction of the fish meal

to 5 per cent. This finding further emphasizes the advisability of supplementing the ration with a little chalk in this final stage of feeding.

*Phosphoric acid balances.* The results appertaining to phosphorus retention during the metabolism experiments are shown in Table IX.

Table IX. *Summary of phosphate balances*

Period	No. of pig and sex	Mean L.W. during period lb.	Diet	P <sub>2</sub> O <sub>5</sub> in daily ration g.	Mean P <sub>2</sub> O <sub>5</sub> eliminated daily		Mean daily P <sub>2</sub> O <sub>5</sub> balance g.	Mean daily N balance g.
					In urine g.	In faeces g.		
1	{1405 H.	48	N.P.	14.99	2.70	6.52	5.77	11.95
	{1410 G.	50	H.P.	15.85	3.29	5.97	6.59	13.66
2	{1406 G.	67.8	N.P.	23.27	6.04	11.47	5.76	12.90
	{1404 H.	67	H.P.	24.62	5.74	13.32	5.56	11.52
3	{1405 H.	87.8	N.P.	31.51	6.04	17.86	7.61	14.38
	{1410 G.	88.5	H.P.	33.32	7.74	17.60	7.98	16.11
4	{1406 G.	107	N.P.	30.51	8.86	16.28	5.37	14.53
	{1404 H.	102.5	H.P.	32.52	8.27	20.49	3.76	9.63
5	{1405 H.	133.5	N.P.	36.51	6.90	20.87	8.74	14.50
	{1410 G.	133.8	H.P.	38.89	9.46	20.67	8.76	16.61
6	{1405 H.	163.5	N.P.	34.15	8.68	19.92	5.55	12.94
	{1410 G.	167	H.P.	36.10	9.84	19.38	6.88	16.58
7	{1406 G.	189	N.P.	39.02	12.44	20.50	6.08	14.86
	{1404 H.	189	H.P.	41.33	10.40	23.30	7.63	14.08

The figures in Table IX suggest that the rate of phosphorus retention in the bacon pig remains fairly constant from weaning to slaughter at about 200 lb. live weight. There was no marked fall in the balances during periods 6 and 7 such as was noted in the lime balances, this difference being due to the fact that the reduction of the white fish meal from 10 to 5 per cent did not affect the phosphate intake in the same degree as the lime intake, owing to the richness of barley meal and weatings in phosphoric acid.

A general consideration of the balance results distinctly suggests that the requirements of the bacon pig for protein, lime and phosphate do not fall off during growth from weaning to slaughter live weight. The pig at 200 lb. live weight is still to be looked on as an immature and quickly-growing animal with a high requirement for such constructive materials as minerals and protein, and the feeding of the bacon pig from 150 to 200 lb. live weight must accordingly be based on a proper appreciation of this finding.

*Chlorine balances.* The essential figures in this connexion are given in Table X.

The outstanding feature of the figures in Table X is the low rate of retention of chlorine by the pigs at almost every stage of growth. Only in

Table X. *Summary of chlorine balances*

Period	No. of pig and sex	Mean L.W. during period lb.	Diet	Cl in daily ration g.	Mean Cl eliminated daily		Mean daily Cl balance g.	Mean daily N balance g.
					In urine g.	In faeces g.		
1	{1405 H.	48	N.P.	2.26	1.97	0.17	0.12	11.95
	{1410 G.	50	H.P.	2.19	1.56	0.14	0.49	13.66
2	{1406 G.	67.8	N.P.	3.52	3.26	0.31	-0.05	12.90
	{1404 H.	67	H.P.	3.40	3.09	0.29	0.02	11.52
3	{1405 H.	87.8	N.P.	4.76	4.03	0.46	0.27	14.38
	{1410 G.	88.5	H.P.	4.60	3.66	0.44	0.50	16.11
4	{1406 G.	107	N.P.	4.85	4.52	0.22	0.11	14.53
	{1404 H.	102.5	H.P.	4.67	4.57	0.24	-0.14	9.63
5	{1405 H.	133.5	N.P.	5.80	3.42	0.49	1.89	14.50
	{1410 G.	133.8	H.P.	5.58	3.34	0.45	1.79	16.61
6	{1405 H.	163.5	N.P.	5.61	4.66	0.59	0.36	12.94
	{1410 G.	167	H.P.	5.34	4.71	0.46	0.17	16.58
7	{1406 G.	189	N.P.	6.41	5.97	0.47	-0.03	14.86
	{1404 H.	189	H.P.	6.11	5.14	0.46	0.51	14.08

period 5 (liveweight of pigs = 133 lb.) were the animals retaining chlorine at an appreciable rate.

The low output of chlorine in the faeces suggests that the chlorine of the food, as well as that derived from the HCl of the gastric secretion, is efficiently absorbed from the intestinal tract; but the relatively large losses in the urine show that much of the chlorine thus absorbed is not actually retained in the body. This is perhaps surprising in view of the power usually attributed to the animal organism of conserving this constituent. It will be noted, however, that an amount of chlorine equal to that in the HCl of the gastric secretion together with a small proportion of the food chlorine is safeguarded against loss in the excreta. It can only be concluded, taking into account the small amounts retained in relation to the actual supply, that the demands for chlorine from the food are not very large, and that the rations ordinarily used in practice, provided they contain white fish meal or the usual amounts of the chalk-salt supplement, are capable of supplying the animal's requirements for this constituent.

#### MINERAL BALANCES IN RELATION TO LEVEL OF PROTEIN INTAKE

No evidence is available from the results of the metabolism trials to warrant the conclusion that the mineral metabolism was consistently affected by the higher plane of protein supply. The average results recorded in Table XI reveal no striking differences in mineral retention that might be correlated with differences in the level of protein intake,

but it would obviously be necessary to experiment with many more animals than could be used in the present trials before a decisive conclusion on this matter could be arrived at.

Table XI. *Summary of daily mineral balances (mean values over whole experiment)*

	Normal-protein diet				High-protein diet			
		CaO g.	P <sub>2</sub> O <sub>5</sub> g.	Cl g.		CaO g.	P <sub>2</sub> O <sub>5</sub> g.	Cl g.
Comparison 1	1405 H.	7.70	6.92	0.66	1410 G.	7.89	7.55	0.74
Comparison 2	1406 G.	7.07	5.74	0.01	1404 H.	5.85	5.65	0.13

#### WHITE FISH MEAL AND BACON TAIN

Since the pigs in the experiment were receiving 5 per cent of white fish meal right up to the day before slaughter, an opportunity thus presented itself of ascertaining whether such feeding can be responsible for a fishy taint in the cured bacon. On this matter there still exists considerable diversity of opinion. Rashers of bacon from both belly and back were accordingly fried under specified conditions and sampled for palatability. The bacon was tested in the mild-cured "green" condition prior to "smoking". The following observations were made:

*Gilt 1410 (high-protein diet).* The smell during frying was slightly unpleasant. The flavour of the fat, although not actually fishy, was unsatisfactory.

*Hog 1405 (normal-protein diet).* The smell during frying was suggestive of kippers, and the flavour of the bacon was slightly fishy.

*Hog 1404 (high-protein diet).* Smell and flavour were quite satisfactory, there being no suggestion of fishiness.

*Gilt 1406 (normal-protein diet).* Smell and flavour were quite satisfactory, with no trace of fishy taint.

Although the observations recorded above are curiously inconsistent, they point to the conclusion that the inclusion of 5 per cent of white fish meal in the finishing rations of bacon pigs may be attended with the risk of producing a slight fishy taint in the cured bacon. Safety lies in discontinuing the use of white fish meal during the final month of feeding.

#### SUMMARY

An investigation has been made, by the method of balance trials, of the utilization of food protein, at different levels of protein intake, by bacon pigs throughout the period of growth from weaning to slaughter.

Information has also been secured relating to the retention of lime, phosphoric acid and chlorine. The main conclusions are as follows:

(1) The young pigs after weaning were able to digest their food with as high an efficiency as was displayed in the later stages of growth. The extra protein in the high-protein rations had little or no effect on the extent to which the food was digested.

(2) No evidence was secured at any stage of the trials suggesting the presence of protein in the urine of the pigs subsisting on the high-protein diet.

(3) The gilts showed a consistently higher rate of nitrogen retention than their brother hogs. This behaviour was manifested even when the protein supply in the gilt's ration was lower than that in the ration of the hog with which it was compared. This more efficient utilization of food protein by the gilts is held to explain the tendency of gilts to give somewhat leaner carcasses than hogs.

(4) Nitrogen retention from the high-protein diet was no higher than from the normal-protein diet, a finding suggesting that the amount of protein in the normal rations is sufficient to meet the demands for the quick growth required by modern standards of bacon production. A very large proportion of the extra protein in the high-protein rations could be accounted for by the extra urea eliminated in the urine of the pigs on these rations. This finding affords a scientific basis for explaining why an increase of the protein supply beyond the levels ordinarily fed in practice leads to no gain in respect of carcass leanness.

(5) The daily retention of nitrogen by the bacon pig remains very much the same throughout the whole period of growth from weaning to slaughter at 200 lb. live weight.

(6) The values for nitrogen retention enabled a correct prediction to be made of the relative leanness of the bacon carcasses, since there was a striking parallelism between the mean nitrogen balances and the figures expressing leanness in the typical rasher submitted to examination.

(7) A consideration of the results of the balance trials suggests that the requirements of the bacon pig for protein, lime and phosphoric acid do not fall off during growth from weaning to slaughter. The pig at 200 lb. live weight is still to be regarded as an immature and quickly growing animal with a high requirement for such constructive materials as protein and minerals.

(8) The demands of the bacon pig for chlorine from the food appear to be small. Rations ordinarily used in good feeding practice are capable of supplying the animal's requirements for this constituent.

(9) No evidence was obtained to warrant the conclusion that the mineral metabolism was affected by the higher plane of protein supply.

(10) The results of palatability tests on the "green" bacon suggest that the retention of 5 per cent of white fish meal in the ration right up to slaughter may be attended with the risk of production of slight fishy taint.

The writers, in conclusion, take this opportunity of acknowledging the help of Messrs V. Thurlbourn and C. Bendall, in whose hands the care of the experimental animals was placed throughout the trials.

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# INVESTIGATIONS ON THE ROOT NODULE BACTERIA OF LEGUMINOUS PLANTS

## XX. EXCRETION OF NITROGEN IN ASSOCIATED CULTURES OF LEGUMES AND NON-LEGUMES

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(With Plate X and Six Text-figures)

ASSOCIATED cultures of legumes and non-legumes have been employed in ordinary farming practice since ancient times, as in such cultures the non-legumes have generally been found to develop better than when grown alone. However, the underlying reason for this beneficial effect has not been clearly understood. Lipman (1912) was probably the first worker to attempt an elucidation of this problem by direct experiments with pot cultures: His results, though in part conflicting, led him to the conclusion that inoculated legumes do somehow furnish nitrogenous food for the non-legumes. Lipman's findings were, however, not conclusive as proof for an excretion. Virtanen's (1929) first experiments with pot cultures showed that oats grew excellently in association with inoculated peas in N-free quartz sand. An excretion of nitrogen was therefore held to be probable. However, in ordinary pot cultures, where the action of various foreign micro-organisms is not excluded, the results may always be rendered valueless through the presence of free-living nitrogen-fixers which thrive particularly well in the rhizosphere. Beijerinck (1908) and others have shown long ago that bacterial life is indeed greatly enhanced in the immediate proximity of the roots of leguminous plants. Under such conditions the nitrogen taken up by the non-legumes is not necessarily derived from the legume nodules but might be fixed by the non-symbiotic organisms. The literature (up to 1934) on the derivation of the nitrogen in associated growth has been reviewed by Nicol (1934).

The first conclusive proof for the excretion of considerable quantities of nitrogenous compounds from the nodules<sup>1</sup> of inoculated legumes was presented by Virtanen & v. Hausen (1931a). The work necessitated the use of "sterile" cultures containing only the desired nodule bacteria,

<sup>1</sup> The excretion of the nitrogenous compounds has been definitely shown to occur from the nodules and not from the roots (Virtanen *et al.* 1936b).

"foreign" micro-organisms being excluded. Vetches, growing on a pumice medium which was moistened with sterile nutrient solution, excreted into the medium over 20 per cent of all the nitrogen fixed. Already at this stage we were able to show that the excreted substances consisted of organic N-compounds, and contained amino-nitrogen.

The use of an improved sterile culture system made it possible to investigate the excretion process from various angles (see Pl. X, fig. 1). Virtanen *et al.* (1933) extracted the excreted compounds from quartz sand cultures of inoculated peas with hot water and found them to consist chiefly of free amino-acids. Subsequently we found that a cold-water extraction of the sand yields almost exclusively amino-acids (Virtanen & Laine, 1935), with very little (1-2 per cent) oxime-N (Virtanen & Laine, 1936).

Table I. *The chemical nature of the N-compounds extracted with water from sterile sand cultures of peas*

	Hot-water extract	Cold-water extract
Amino-N, % of total N	77.4*	98.8†
Ammonia-N, % of total N	0	—
Amido-N, % of total N	3.0	—
Volatile bases-N, % of total N	2.7	—
Melanin-N, % of total N	2.1	—

\* Shaken in the van Slyke apparatus for 5 min. The percentage of amino-N would undoubtedly have been somewhat higher, had the mixture been shaken for 30 min.

† Shaken for 30 min.

The other nitrogenous compounds obtained by the hot-water extraction were obviously formed from some amino-acid during the extraction, since the cold-water extract contained almost exclusively amino-acids.

Further work on the excreted amino-acids showed that they consisted of two compounds: *l*-aspartic acid and an amino-acid which was precipitated by phosphotungstic acid. Since cystine, histidine, and aromatic amino-acids were absent, it is probable that this fraction consisted of a diamino-acid (lysine?). So far we have not succeeded in demonstrating the presence of lysine.<sup>1</sup> The aspartic acid-N at the commencement of flowering in the different experiments formed about 50 per cent of the total N-content of the extract.

In three experiments in which peas in sand culture were inoculated with the three strains (one at a time) of pea nodule bacteria H X, H IV, and H XV, the aspartic acid-N at the beginning of flowering was respectively 51.0, 45.7 and 51.0 per cent of the total N in the extract.

<sup>1</sup> See the note on p. 589.



Owing to the limited amount of experimental material, it is not possible to say how far this fairly constant relation holds true. The amount of amino-acid-N precipitable by phosphotungstic acid varied in different extracts from 40 to 46 per cent of the total N.

Our recent research on the chemical nature of the excreted N-compounds is closely associated with our earlier work (Virtanen, 1929) which showed that in sterile cultures the legumes utilize readily certain amino-acids, especially aspartic acid. Thus, for instance, red clover grew better on aspartic acid-N than on nitrate-N while, on the other hand, it also grew better when inoculated with an efficient strain of the nodule organism than when not inoculated but supplied with nitrate nitrogen. These results led Virtanen to the conclusion that the legumes receive their nitrogen nutrition from the nodules in the form of amino-acids. The demonstration of an excretion of amino-acids from the nodules gave support to this view, as it was reasonable to expect that the plant would take up from the nodules N-compounds similar to those transferred to the medium.

Experiments with associated cultures of inoculated peas and barley under sterile conditions showed that the barley utilizes at least part of the excreted amino-acids, since in such cultures it grew fairly well on an otherwise N-free medium (Virtanen *et al.* 1933). At the conclusion of the experiments, the sand still contained considerable quantities of nitrogen, so that the barley obviously could not utilize all the excreted N-compounds. Our earlier experiments had shown that barley utilizes aspartic acid very poorly in media where this amino-acid is the only source of nitrogen. Consequently we deduced that in associated cultures barley utilizes the excreted amino-acid precipitable by phosphotungstic acid. We have already shown (Virtanen *et al.* 1937) that the percentage content of aspartic acid in the sand (total sand-N basis) is greater in associated cultures of peas and barley than in cultures of peas alone, showing that the other amino-acid has been utilized by the barley more than aspartic acid.

Associated cultures of legumes and non-legumes, under conditions which exclude the breakdown of the excreted amino-acids by foreign micro-organisms, thus present the interesting picture that one of the excreted amino-acids is mainly utilized by the non-legume while the other amino-acid serves as an excellent N-source for the legume. It is probable that the legume also takes up the excreted amino-acids through the roots, particularly during the later stages when the function of the nodules becomes weaker. Part of the aspartic acid present in the medium will then be utilized by the legume.

## I. ASSOCIATED GROWTH IN STERILE CULTURES

The experimental technique employed in our work on the associated cultures was the same as in experiments with peas alone, except that the inoculated peas and the non-legumes were now grown in the same culture flask. The transfer of the non-legume seedling into the culture flask was carried out in a similar manner to that of the pea seedling. A three-necked Woulff's bottle was generally used as culture flask. The pea grew up through one neck, the non-legume through another. The nutrient solution consisted of the modified (N-free) Hiltner's solution generally used in our sterile experiments. For a detailed description of our sterile culture technique, reference is made to Virtanen *et al.* (1937).

We give below some of the results of our experiments with associated cultures of peas and different non-legumes. The variety of pea employed was the Torsdag pea from Svalöf. One pea seed contained, on an average, 7.0 mg. N, while the average N-content of the seeds of barley, oats and wheat employed by us was 0.7 mg.

*Associated cultures of peas and barley*

Table II

3 l. Woulff's bottles; 4.4 kg. quartz sand; 2 l. nutrient solution; pH 6.5. The initial N-content of the sand was 3.0 mg. per kg. One pea (Torsdag) and one barley (Lapland variety) in each flask. Period of growth: 2 May–9 June 1932.

No. of culture	Inoculation	Dry weight (g.)		N (mg.)*			Total fixed N mg.	Extent of excretion %	N transferred to barley, % of excreted N
		Pea	Barley	Pea	Barley	Sand			
1	Strain H IV	1.462	1.791	41.8	32.3	89.0	163.1	74.3	26.6
2	"	2.048	1.660	55.8	21.7	77.4	154.9	64.0	21.9
3	"	1.382	1.432	42.6	24.4	93.9	160.9	73.5	20.6
4	"	2.400	0.655	60.7	13.0	67.6	141.3	57.0	16.1
5	None; control	0.297	0.063	6.4	0.7	10.0	—	—	—

\* The values for the inoculated cultures are given after subtraction of the corresponding values for the uninoculated controls.

Table III

3 l. Woulff's bottles; 4.4 kg. quartz sand; 2 l. nutrient solution, pH 6.5. Initial N-content of sand 3.6 mg. per kg. Period of growth: 15 June–25 July 1934.

*Exp. 1.* 2 inoculated (strain H X) peas (Torsdag) + 3 barley (Binder).

*Exp. 2.* 2 inoculated (strain H X) peas (Torsdag) + 4 barley (Binder).

*Exp. 3.* 2 uninoculated peas (Torsdag) + 3 barley (Binder) (control).

Exp.	Dry weight (g.)		N (mg.)*			Total excreted N mg.	Extent of excretion %	N transferred to barley, % of excreted N
	Peas	Barley	Peas	Barley	Sand			
1	3.605	2.409	57.0	45.2	27.5	72.7	56.4	62.2
2	1.230	1.657	8.3	16.7	24.2	40.9	83.1	40.8
3	0.514	0.359	15.4	5.1	8.6	—	—	—

\* The values for Exps. 1 and 2 are given after subtraction of the corresponding values for Exp. 3 (7.7 mg. N per pea, 1.7 mg. N per barley and 8.6 mg. N for the sand).

Table IV

1 l. suction flasks; 1.4 kg. sand; 1 l. nutrient solution, pH 6.5. Initial N-content of sand 3.6 mg. per kg. Period of growth: 15 June–25 July 1934.

*Exp. 1.* 1 inoculated (strain H X) pea (Torsdag) + 1 barley (Binder).

*Exp. 2.* 2 inoculated (strain H X) peas (Torsdag) + 4 barley (Binder).

*Exp. 3.* 2 uninoculated peas (Torsdag) + 4 barley (Binder).

Exp.	Dry weight (g.)		N (mg.)*			Total fixed N mg.	Extent of excretion %	N transferred to barley, % of excreted N
	Peas	Barley	Peas	Barley	Sand			
1	2.295	0.738	40.6	13.6	9.5	63.7	36.3	59.3
2	2.603	2.672	39.7	39.1	12.6	91.4	56.6	75.4
3	0.487	0.249	13.8	4.0	4.7	—	—	—

\* The values for Exps. 1 and 2 are given after subtraction of controls (6.9 mg. N per pea, 1.0 mg. N per barley and 4.7 mg. N for the sand).

Table V

*Associated cultures of peas and oats*

3 l. Woulff's bottles; 4.4 kg. sand; 2 l. nutrient solution, pH 6.5. Initial N-content of sand 3.6 mg. per kg. Period of growth: 15 June–25 July 1934.

*Exp. 1.* 2 inoculated (H X) peas (Torsdag) + 2 oats (Guldregn II).

*Exp. 2.* 1 inoculated (H X) pea (Torsdag) + 2 oats (Guldregn II).

*Exp. 3.* 2 uninoculated peas (Torsdag) + 2 oats (Guldregn II).

Exp.	Dry weight (g.)		N (mg.)*			Total fixed N mg.	Extent of excretion %	N transferred to oats, % of excreted N
	Peas	Oats	Peas	Oats	Sand			
1	2.912	1.423	45.4	16.5	26.5	88.4	48.6	38.4
2	2.051	1.370	35.6	17.2	23.0	75.8	53.0	42.8
3	0.589	0.367	12.0	3.5	9.5	—	—	—

\* The values for Exps. 1 and 2 are given after subtraction of controls (6 mg. N per pea, 1.75 mg. N per oat and 9.5 mg. N for the sand).

Table VI

*Associated cultures of peas and wheat*

3 l. Woulff's bottles; 4.4 kg. sand; 2 l. nutrient solution, pH 6.5. Initial N-content of sand 3.6 mg. per kg. Period of growth: 15 June–27 July 1934.

*Exp. 1.* 2 inoculated (H X) peas (Torsdag) + 2 wheats (Aurora).

*Exp. 2.* 2 uninoculated peas (Torsdag) + 2 wheats (Aurora).

Exp.	Dry weight (g.)		N (mg.)*			Total fixed N mg.	Extent of excretion %	N transferred to wheat, % of excreted N
	Peas	Wheats	Peas	Wheats	Sand			
1	2.048	1.413	37.6	30.8	28.0	96.4	61.0	52.4
2	0.436	0.203	15.3	1.4	12.2	—	—	—

\* After subtraction of the control.

The results of the sterile associated culture experiments in Tables II-VI show that:

(1) The extent of excretion rose with increasing ratio of non-legumes to legumes. This is clearly seen from all the experiments.

(2) The growth of peas suffered when the number of non-legumes rises. Thus, Table III shows that when two peas were grown together with four barley plants, the peas received from the nodules only 8.3 mg. N, while the barley received 16.7 mg., a further 24.2 mg. N being found in the sand. In this experiment the extent of excretion amounted to 83.1 per cent.

(3) Oats and wheat took up about one-half or less of the excreted nitrogen. On the other hand, in some cases where the cultures contained more than one barley plant for each pea plant, the barley appropriated up to 75 per cent of the excreted nitrogen. Considering that in our earlier sterile culture experiments with different N-sources the barley did not grow on aspartic acid-N, which constitutes about 50 per cent of the total excreted N, the above results are rather unexpected. It is possible that the barley has now utilized also aspartic acid to some extent.<sup>1</sup>

As mentioned previously, the percentage content of aspartic acid-N, in proportion to the amino-acid-N precipitable by phosphotungstic acid, rose when barley was grown together with inoculated peas. This was demonstrated by the following experiment:

Inoculated peas and barley were grown together in four 3 l. Woulff's bottles, each containing 4 kg. quartz sand. The plants were harvested as soon as the peas flowered. The sand was washed off from the bottles with some water, whereupon the roots were carefully removed and the four lots of sand combined. Total-N was then determined from numerous samples of the sand. The bulk of the sand was thoroughly extracted with water in two portions in the following manner: Both portions were washed ten times, each time with 3 l. tap water containing 100 ml. 0.1 *N* H<sub>2</sub>SO<sub>4</sub>. Each 3 l. portion was passed through the sand ten times with vigorous shaking. The extracts were treated with toluene to prevent contamination. The combined extracts (60 l.) were concentrated in a vacuum distillation apparatus with automatic filling to a volume of

<sup>1</sup> Virtanen & Laine have recently (*Suomen Kemistilehti* B, Jan. 1937) shown that the amino-acid fraction precipitable with phosphotungstic acid does not contain lysine, but  $\beta$ -alanine, which is formed from aspartic acid by legume bacteria. The above observations that the barley in sterile system in some cases has utilized considerably over 50 per cent of the excreted nitrogen do not necessarily mean that the barley would have utilized aspartic acid as  $\beta$ -alanine has continuously been formed from aspartic acid during the experiment.

## 590 *The Root Nodule Bacteria of Leguminous Plants*

250 ml. The rate of distillation was 5 l. per 6 hr. The precipitate formed was filtered off and washed, and the washings were added to the extract. The insoluble residue contained only an insignificant amount of nitrogen.

The final extract contained 75.5 per cent of the total excreted N present in the sand. Amino-N was determined according to van Slyke (30 min. shaking) and found to amount to 89.6 per cent of the total N in the extract. Dicarboxylic acid-N, determined according to Foreman, was 65.6 per cent, while aspartic acid-N, determined with the enzyme aspartase, was 64.5 per cent of the total N in the extract. The dicarboxylic acid present was thus exclusively aspartic acid. The results are given in Table VII.

Table VII

3 l. Woulff's bottles, 4 kg. quartz sand; 2 l. nutrient solution. Initial N-content of sand 3.8 mg. per kg. Period of growth: Exps. 1 and 2, 15 August–1 October 1935; Exps. 3 and 4, 15 October–25 November 1935.

*Exp. 1.* 2 inoculated (H X) peas (Torsdag) + 2 barleys (Binder).

*Exp. 2.* 1 inoculated (H X) pea (Torsdag) + 1 barley (Binder).

*Exp. 3.* 1 inoculated (H X) pea (Torsdag) + 2 barleys (Binder).

*Exp. 4.* 1 inoculated (H X) pea (Torsdag) + 1 barley (Binder).

*Exp. 5.* 1 uninoculated pea (Torsdag) + 1 barley (Binder).

Exp.	Dry weight (g.)		N (mg.)*		Total N (mg.)*			Extent of excretion %	N transferred to barley, % of total excreted N
	Peas	Barley	Peas	Barley	Peas	Barley	Sand		
1	4.515	2.646	93.7	56.7	261.9	131.7	153.2	52.2	46.2
2	3.746	0.946	110.9	23.2					
3	1.620	1.532	27.3	35.1					
4	1.557	0.680	30.0	16.7	Total fixed N = 545.8 mg.			—	—
5	0.261	0.070	7.0	0.7	—	—	15.0		

\* The values for Exps. 1–4 are given after subtraction of controls.

### *Composition of the water extract (in per cent of the total N in the extract)*

Amino-N	...	...	89.6 %
Dicarboxylic acid-N	...	...	65.6 ,
Aspartic acid-N	...	...	64.5 ,

The barleys have thus taken up 46.2 per cent of the total excreted N. In view of the fact that the aspartic acid-N in the extract amounted to about 65 per cent of the total extract-N, the corresponding figure in cultures of peas alone being generally about 50 per cent, it will be seen that the percentage of aspartic acid has increased in the medium of the associated cultures.

Quite recently we obtained the following results in an experiment with associated cultures of peas and barley (Table VIII).

Table VIII

3 l. Woulff's bottles, 4 kg. quartz sand; 2 l. nutrient solution. Initial N-content of sand 3.8 mg. per kg. Variety of pea: Torsdag. Variety of barley: Binder. Period of growth: 21 July–19 October 1936.

*Exp. 1.* 1 inoculated (H X) pea + 1 barley.

*Exp. 2.* 1 uninoculated pea + 1 barley.

Exp.	Dry weight (g.)		N (mg.)*			Total fixed N mg.	Extent of excretion %	N transferred to barley, % of total excreted N	NH <sub>3</sub> -N in the water extract, % of total N	Aspartic acid-N, % of total N in extract
	Peas	Barley	Peas	Barley	Sand					
1	5.206	4.223	81.5	35.3	29.7	146.5	44.5	54.3	99.6	59.3
2	0.420	0.403	5.7	4.3	16.8	—	—	—	—	—

\* Controls subtracted from the values for inoculated cultures.

## II. NON-STERILE POT CULTURES

Our investigations with sterile cultures had thus shown that nitrogenous compounds are excreted into the solid medium from the nodules of leguminous plants. Hence it might be concluded that even in ordinary pot cultures a benefit to the non-legumes accrues from this excretion. The main difference between pot cultures and sterile cultures is that in the former the excreted amino-acids are susceptible to decomposition by the stray micro-organisms present in the medium, so that the non-legumes would receive at least part of their nitrogenous food in the form of ammonia or nitrate. We refer here to our earlier work on associated cultures of peas and oats, and of red clover and *Alopecurus*, as well as mixtures of white clover and *Dactylis* (Virtanen & v. Hausen, 1930a, 1931b).

### (1) Quartz sand cultures

The experimental procedure was the following: Unglazed earthenware pots were used as culture vessels. The size of the pots will be mentioned in the description of the different experiments. The solid medium consisted of quartz sand, the N-content of which varied from 2.6 to 3.7 mg. per kg. in different years. The sand was sterilized in the autoclave in order to control nodulation. The plants could not develop in this sand without inoculation or artificial N-supply. The composition of the N-free watering solution (modified Hiltner's solution) was the following: Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 0.25 g., CaSO<sub>4</sub> · 2H<sub>2</sub>O 0.25 g., MgSO<sub>4</sub> · 7H<sub>2</sub>O 0.394 g., KCl 0.25 g., FeCl<sub>3</sub> (5 per cent solution) 3 drops. Distilled water to 1000 ml. The pH of the watering solution was 6.5. The pH of the quartz sand was maintained at 6.5 throughout the period of growth. At the conclusion of the experiments the plants were clipped and the sand was poured on a sheet

## 592 *The Root Nodule Bacteria of Leguminous Plants*

of hard paper. The roots of the legumes and the non-legumes were removed separately and dried and analysed for nitrogen according to Kjeldahl's method. The peas and non-legumes were likewise dried separately, finely ground, and the total N was determined from duplicate samples of each. To determine the N-content of the sand, it was dried and thoroughly mixed, and three composite samples of 100 g. each were digested by Kjeldahl's method. The deviations between the individual determinations did not exceed 10 per cent, being generally less than 5 per cent.

### *Associated pot cultures of peas and oats in quartz sand*

One series of experiments, carried out in the summer of 1929, has been previously recorded (Virtanen & v. Hausen, 1930*b*). It was ascertained that the growth of both peas and oats suffered, obviously from lack of nitrogen, when the ratio of oats to peas exceeded 2. Since the total yields of nitrogen are not clearly stated in the paper referred to, we give the exact figures in Table IX. The variety of pea was Concordia and that of oats Argus. The peas were inoculated with strain H IV. Each pot contained 30 kg. of dry quartz sand, 30 plants being grown in each pot. The figures for the yields include also the roots. All values are given without subtraction of the controls.

Table IX

	Inoculated										Uninoculated	
	15 peas + 15 oats		10 peas + 20 oats		8 peas + 22 oats		6 peas + 24 oats		5 peas + 25 oats		15 peas + 15 oats	
	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats
Dry matter (g.)												
All plants	42.48	30.72	26.68	37.72	20.98	34.23	10.38	36.29	8.85	36.20	1.32	4.12
Per plant	2.83	2.05	2.67	1.89	2.62	1.56	1.73	1.51	1.77	1.45	0.09	0.28
N (mg.)												
All plants	1308.0	645.0	966.0	896.0	608.0	726.0	277.2	652.4	239.0	675.0	90.0	48.0
Per plant	87.2	43.0	96.6	44.8	76.0	33.0	46.2	27.6	47.8	27.0	6.0	3.2

The average nitrogen contents of single plants in this experiment are presented graphically in Text-fig. 1.

The results show clearly that with increasing ratio of oats to peas the growth of the pea was seriously impaired and its N-content fell. The fact that the oats were less seriously affected suggests that in the competition between the legumes and the non-legumes the more numerous oats deprived the peas of their nitrogenous food.

In the next experiment (Table X) the growth of peas was already greatly impaired when the ratio of oats to peas changed from 1 to 2.

This effect was not discernible in the experiments summarized in Table IX. The difference is obviously ascribable to the fact that in the later experiment 24 plants were grown in 9 kg. sand, whilst in the earlier experiments (Table IX) 30 plants grew in 30 kg. sand.

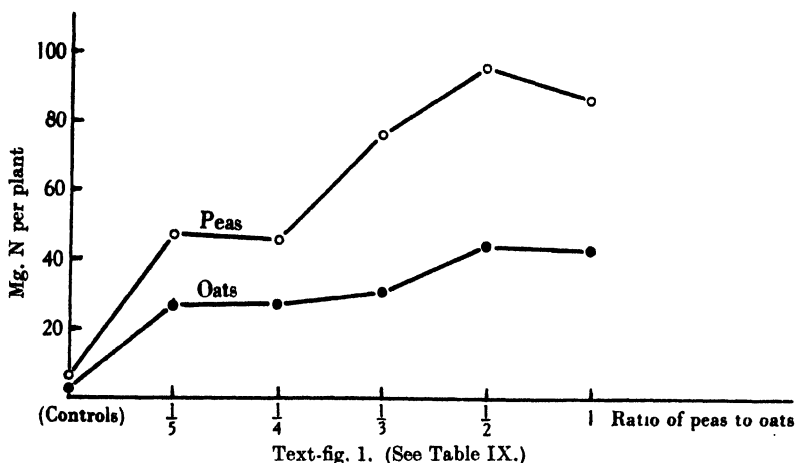


Table X

Earthenware pots, 9 in. diam.; 9 kg. dry quartz sand; pH 6.5; 24 plants in each pot. Variety of oats: Guldregn II. Peas (Torsdag) inoculated with strain H X. Period of growth: 4 August–19 October 1934. The yields include also the roots.

	Inoculated				Uninoculated	
	12 peas + 12 oats		8 peas + 16 oats		12 peas + 12 oats	
	Peas	Oats	Peas	Oats	Peas	Oats
Dry matter (g.)						
All plants	31.59	26.31	15.96	33.30	2.97	1.77
Per plant	2.63	2.19	2.00	2.08	0.25	0.15
N (mg.)						
All plants*	821.4 (717.8)	292.9 (257.4)	379.9 (310.8)	408.3 (361.0)	103.6	35.5
Per plant	68.4	24.4	47.5	25.5	8.6	3.0
* N, % of dry matter	2.60	1.11	2.37	1.23	3.44	2.00

\* The figures in brackets were obtained after subtraction of the corresponding values for controls.

In order to compare the effect of a varied ratio of oats to peas on the yield of both species in inoculated cultures, without mineral nitrogen, and in *uninoculated cultures growing on nitrate nitrogen* we carried out several series of experiments with the same technique except that in the uninoculated cultures the watering solution contained 3.2 g.  $\text{NH}_4\text{NO}_3$  per 10 l. The results of these experiments are presented in Tables XI



## 594 *The Root Nodule Bacteria of Leguminous Plants*

and XII and the average nitrogen contents of single plants are presented graphically in Text-figs. 2 and 3. The same controls served for all three experiments.

Table XI

Earthenware pots, 9 in. diam.; 9 kg. dry quartz sand, pH 6.5; 14-15 plants in each pot. Period of growth: 15 June-22 August 1936. Oats: Guldregn II; peas inoculated with strain H X. The yields do not include the roots. Controls not subtracted.

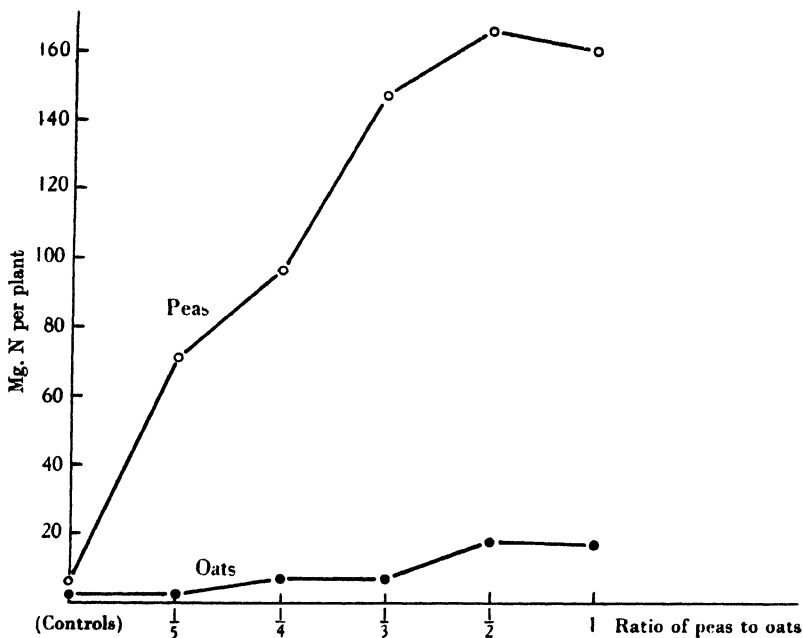
	Inoculated										Uninoculated	
	7 peas + 7 oats		5 peas + 10 oats		4 peas + 11 oats		3 peas + 12 oats		2 peas + 12 oats		7 peas + 7 oats	
	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats
Dry matter (g.)												
All plants	41.16	8.80	29.32	12.23	21.59	8.82	10.49	7.41	4.77	5.52	1.45	1.15
Per plant	5.88	1.26	5.86	1.22	5.40	0.80	3.50	0.62	2.39	0.46	0.21	0.16
N (mg.)												
All plants	1123.4	122.9	826.5	185.7	588.2	79.5	292.0	87.0	145.1	50.8	43.2	15.8
Per plant	160.5	17.6	165.3	18.6	147.0	7.2	97.3	7.2	72.6	4.2	6.2	2.3
N, % of dry matter	2.73	1.40	2.82	1.52	2.72	1.22	2.78	1.17	3.04	0.92	2.98	1.37

Table XII

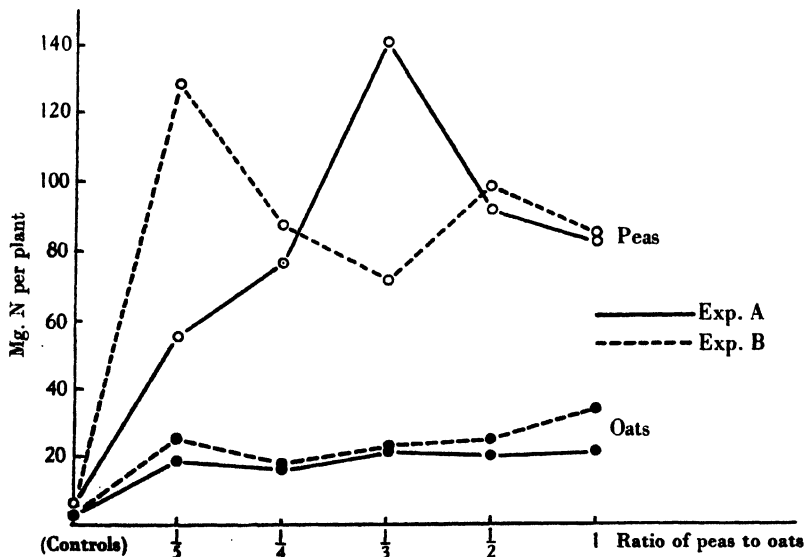
Earthenware pots, 9 in. diam.; 9 kg. dry quartz sand; pH 6.5; 14-15 plants in each pot. Period of growth: 15 June-12 August 1936. Oats: Guldregn II. The yields do not include the roots. Controls not subtracted.

	Uninoculated $\text{NH}_4\text{NO}_3$ -cultures									
	7 peas + 7 oats		5 peas + 10 oats		4 peas + 11 oats		3 peas + 12 oats		2 peas + 12 oats	
	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats	Peas	Oats
Exp. A										
Dry matter (g.)										
All plants	21.53	16.00	20.72	20.62	21.25	18.53	10.45	20.95	6.08	25.74
Per plant	3.08	2.29	4.14	2.06	5.31	1.68	3.48	1.75	3.04	2.15
N (mg.)										
All plants	583.5	155.3	464.3	206.0	566.0	240.4	234.5	203.0	112.4	230.3
Per plant	83.8	22.2	92.9	20.6	141.5	21.9	78.2	17.0	56.2	19.2
N, % of dry matter	2.72	1.03	2.24	1.00	2.66	1.30	2.24	0.97	1.85	0.90
Exp. B										
Dry matter (g.)										
All plants	26.60	12.29	17.11	15.73	13.39	13.44	8.74	19.72	11.67	19.69
Per plant	3.80	1.76	3.42	1.57	3.35	1.22	2.91	1.64	3.89	1.64
N (mg.)										
All plants	603.7	240.4	496.7	248.6	288.2	259.4	266.6	211.4	257.6	307.7
Per plant	86.2	34.3	99.3	24.9	72.1	23.6	88.9	17.6	128.8	25.6
N, % of dry matter	2.27	1.95	2.90	1.57	2.15	1.93	3.06	1.07	2.20	1.56

An examination of the results obtained with the nitrate cultures shows that the growth of oats was almost equal in all the different cultures, in spite of the fact that the ratio of oats to peas was varied from 1 to 6. The poorer growth of oats noted in the inoculated cultures with increasing ratio of oats to peas is therefore obviously ascribable to lack of nitrogen.



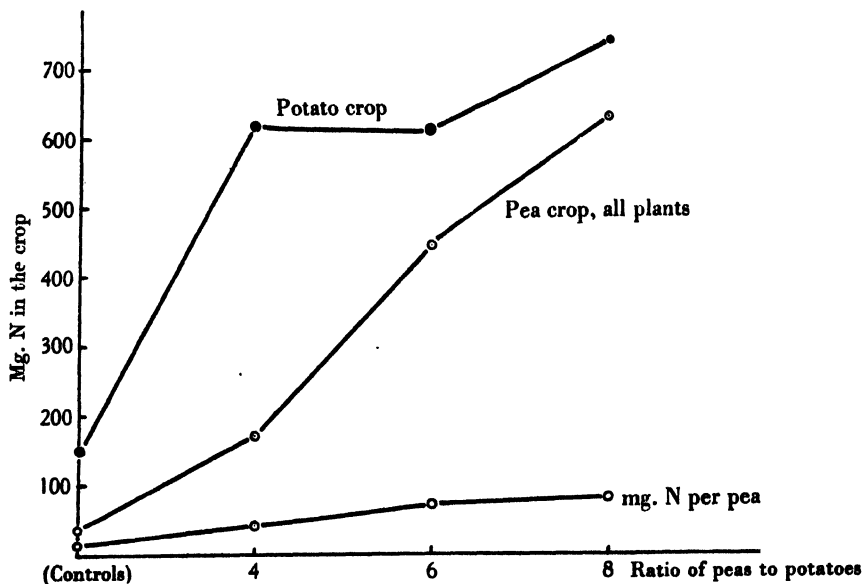
Text-fig. 2. (See Table XI.)



Text-fig. 3. Uninoculated cultures of peas and oats growing on nitrate nitrogen (see Table XII).

## 596 *The Root Nodule Bacteria of Leguminous Plants*

No regular trend can be noted as regards the effect of a varying ratio of oats to peas on the growth of the peas in the nitrate cultures. True, the dry yields show considerable and irregular variations, whereas the mean values from the two series are fairly even throughout. Hence it can be concluded that the growth of the peas has been practically equal in the mixtures possessing different ratios. Consequently, the striking impairment of the growth of peas, noted in the inoculated cultures with more than two oats per pea plant, must be due to the fact that the oats



Text-fig. 4. (See Table XV.)

actually deprived the peas of their nitrogenous food and possibly also of utilizable carbon compounds. In other words, the excretion from the nodules rises to such an extent that the peas themselves came to suffer from lack of nitrogen.

In the experiments carried out between 15 June and 12 August 1936, the oats generally gave considerably higher yields in the nitrate cultures than in the corresponding inoculated, nitrate-free cultures. On the other hand, in the 1929 experiments (Table IX), the inoculated cultures gave excellent yields of oats. In best cases the oats contained from 43 to 44.8 mg. nitrogen per plant. It is pointed out here that the bacterial strains used for inoculating the peas in the 1929 and 1936 experiments were not the same (H IV and H X, respectively).

Table XIII

*Associated cultures of peas and barley in quartz sand*

Earthenware pots, 9 in. diam.; 9 kg. dry quartz sand; pH 6.5; 24 plants in each pot. Peas (Torsdag) inoculated with strain H X. Variety of barley: Binder. Periods of growth: Exp. A: 28 May-23 July 1934; Exp. B: 4 August-19 October 1934. The yields include the roots.

	Exp. A						Exp. B					
	Inoculated			Controls			Inoculated			Controls		
	12 peas	to 12 barley	8 to 16 peas barley	12 to 12 peas barley	12 to 12 peas barley	12 to 12 peas barley	12 to 12 peas barley	8 to 16 peas barley	12 to 12 peas barley	12 to 12 peas barley	12 to 12 peas barley	12 to 12 peas barley
Dry matter (g.)												
All plants	27.49											
Per plant	2.29	7.78	20.84	14.69	1.56	1.31	33.20	11.40	24.98	10.47	2.63	1.60
N (mg.)		0.65	2.61	0.92	0.13	0.11	2.77	0.95	3.12	0.65	0.22	0.13
All plants*	811.1	108.3	502.9	206.4	69.4	21.6	866.1	153.3	608.8	124.3	67.2	27.5
Per plant	(741.7)	(86.7)	(456.6)	(177.6)			(798.9)	(125.8)	(564.0)	(87.6)		
	67.6	9.0	62.9	12.8	5.8	1.8	72.2	12.8	76.1	7.7	5.6	2.3
N in sand (mg.)*	93.6		65.1			41.4						
	(52.2)		(23.7)									
Total fixed N (mg.)	880.6		657.5			—						
Excreted N (mg.)†	138.9		201.3			—						
Extent of excretion (%)	15.8		30.6			—						
Barley-N, % of excreted N‡	62.4		88.2			—						

\* The figures in brackets are given after subtraction of the controls.

† N taken up by the barley + N in the sand (over the control).

‡ Refers to transferred N.

### *Associated cultures of peas and wheat in quartz sand.*

Dry matter (g.)

### All plants

Per plant

N (mg.)

IN (mg.) All plants\*

## IV. Practices

Per plant

and

**N in sand (mg.)\***

1970

Total fixed N (mg.)

Excreted N (mg.)†

### Extent of excretion

**N transferred to wh**  
**0% of created N**

[illegible]

\* The figures in brackets are given after subtraction of the controls.

+ N transferred on the wheat + N in the sand (over the control).

Table XV  
*Associated cultures of peas and potato in quartz sand*

Earthenware pots, 9 in. diam.; 9 kg. dry quartz sand; pH 6.5. Variety of potato: Up-to-Date. The peas (Torsdag) were inoculated with strain H X. Each pot contained, besides the peas, one potato plant, grown from a halved potato tuber. These were selected so as to have as nearly as possible the same weight in each pot. The mean N-content of the halved tubers employed was 180 mg. The potatoes and the peas were sown on 22 May 1934. The peas were harvested almost ripe on 4 August, and the potatoes on 5 September, when the roots of peas were all removed from the sand. The yields include also the roots.

	Inoculated						Controls					
	4	to	1	6	to	1	6	to	1	4	to	1
	peas	potato	peas	peas	potato	peas	peas	potato	potato	peas	peas	potato
Dry matter (g.)												
All plants	7.12	39.24*	16.27†	53.32	25.49†	50.68	2.18	11.91	11.91	1.50	11.90	11.90
Per plant	1.78	39.24	2.71	53.32	3.19	50.68	0.36	11.91	11.91	0.38	11.90	11.90
N (mg.)												
All plants	169.1	618.2	444.5	613.9	640.2	729.0	34.7	153.3	153.3	30.4	146.4	146.4
Per plant	42.3	(471.8)	(409.8)	(460.6)	(593.9)	(575.7)	5.8	153.3	153.3	7.6	146.4	146.4
	(34.7)	618.2	74.1	613.9	80.0	729.0						
			(68.3)		(74.2)							
N in sand (mg.)		90.1		93.4		115.5	56.8			66.6		
		(23.5)		(36.6)		(58.7)						
Total fixed N (mg.)§		634.0		907.0		1228.3						
Excreted N (mg.)		495.3		497.2		634.4						
Extent of excretion (%)		78.1		54.8		51.6						
N transferred to potato, % of excreted N		95.3		92.6		90.7						

\* Tops 13.83 g. (330.3 mg. N), tubers and roots 25.41 g. (287.9 mg. N).

† Tops 14.88 g. (196.2 mg. N), tubers and roots 38.44 g. (417.0 mg. N).

‡ Tops 17.51 g. (396.9 mg. N), tubers and roots 33.17 g. (332.1 mg. N).

§ After subtraction of the controls.

|| N transferred on potato + N in the sand (over the control).

## 600 *The Root Nodule Bacteria of Leguminous Plants*

The photograph (Pl. X, fig. 2) shows the cultures before cutting. The main average results for nitrogen *per pot* are illustrated graphically in Text-fig. 4.

The results of the pea-potato cultures compiled in Table XV disclose several interesting facts. First, it will be seen that the potato deprived the peas of their nitrogenous food to a surprising extent. Thus, in the culture of four peas and one potato, the latter took up 3.5 times as much nitrogen as that received by the peas. The peas therefore grew poorly. Each pea plant has received only 34.7 mg. N from the nodules. When six peas were grown together with one potato, the peas grew much better, each plant receiving a doubled amount of nitrogen (68.3 mg.) from the nodules. Although these proportions varied considerably in the different experiments, the general trend is quite clear. It is also shown by the culture experiments in low-nitrogen soil, to be recorded below. Secondly, it is seen that, although in the associated cultures of peas and potatoes the growth of the peas suffered seriously, *the total amount of nitrogen fixed per pea plant was approximately the same in all the cultures if the excreted nitrogen is also taken into account.* This fact is illustrated in Table XVI.

Table XVI

	4 peas + 1 potato	6 peas + 1 potato	8 peas + 1 potato
Total fixed N per pea (mg.)	158.5	151.2	153.5
Dry weight of one pea (g.)	1.78	2.71	3.19

This is a most extraordinary result in view of the fact that the extent of growth of the peas was very different in the different mixtures. It could not be expected that the nodules of the poorly grown peas would have received from their host plants as much carbohydrate material for the amino-acid synthesis as did the nodules of the better-grown peas. Figuratively speaking, it could be said that in the culture of four peas and one potato the legume nodules have fixed nitrogen mainly for the benefit of the potato.

### (2) *Experiments in soil*

These experiments were made with two different kinds of soil: clay soil and sandy loam soil. The soils were impoverished with respect to nitrogen by repeated cropping in the following manner: a layer of soil, some 15 cm. in thickness, was spread in wooden boxes, sufficient limestone being added to bring the pH of the soil to 6.5. Four successive oat crops were then grown in the boxes. The plants were watered with the N-free nutrient solution usually employed in this laboratory. After

Table XVII

*Associated cultures of peas and oats in soil*

*Exp. 1. Clay soil.* 5 kg. dry soil in each pot. Peas: Torsdag; oats: Guldregn II. Peas inoculated with strain H X. 24 plants in each pot. Period of growth: 23 July-10 October 1935. The yields do not include roots.

*Exp. 2. Sandy loam soil.* 7 kg. dry soil in each pot. Otherwise as in Exp. 1.

	Inoculated										Controls			
	Exp. 1					Exp. 2								
	8 to 16 peas	8 to 16 oats	8 to 16 peas	12 to 12 oats	12 to 12 peas	8 to 16 peas	8 to 16 oats	12 to 12 peas	12 to 12 oats	8 to 16 peas	8 to 16 oats	12 to 12 peas	12 to 12 oats	
Dry matter (g.)														
All plants	12.53	6.32	14.74	7.75	28.12	7.78	28.22	8.40	2.65	1.26	5.13	0.60		
Per plant	1.57	0.40	1.84	0.48	2.34	0.65	2.35	0.70	0.33	0.08	0.43	0.05		
N (mg.)														
All plants*	289.7	111.4	385.8	147.7	736.5	193.9	779.2	158.0	77.5	28.3	100.9	10.4		
Per plant	(212.2)	(83.1)	(308.3)	(119.4)	(635.6)	(183.5)	(678.3)	(147.6)	9.7	1.8	8.4	0.9		
Oats-N/peas-N (%)†	36.2	7.0	48.2	9.2	61.4	16.2	64.9	13.2	—	—	—	—		
	39	39	39	29	22									
Dry matter (g.)														
All plants	12.09	11.52	12.71	7.91	29.32	12.49	19.47	17.11	3.67	3.54	5.13	2.57		
Per plant	1.51	0.72	1.59	0.49	2.44	1.04	1.62	1.43	0.46	0.22	0.43	0.21		
N (mg.)														
All plants*	315.0	210.4	358.7	161.4	751.9	200.0	536.5	270.2	107.6	72.3	92.5	48.3		
Per plant	(207.4)	(138.1)	(251.1)	(89.1)	(659.4)	(151.7)	(444.0)	(221.9)	13.5	4.5	7.7	4.0		
Oats-N/peas-N (%)†	39.4	13.2	44.8	10.1	62.7	16.7	44.7	22.5	—	—	—	—		
	67	35	35	23	50									

\* The figures in brackets were obtained after subtraction of the corresponding controls.

† Refers to the transferred N.



Table XV]]]

*Associated cultures of peas and wheat in soil*

*Exp. 1. Clay soil.* 5 kg. dry soil in each pot. Peas: Torsdag; wheat: Aurora. Peas inoculated with strain H X. 24 plants in each pot. Period of growth: 23 July–10 October 1935. The yields do not include roots.  
*Exp. 2. Sandy loam soil.* 7 kg. dry soil in each pot. Otherwise as in Exp. 1.

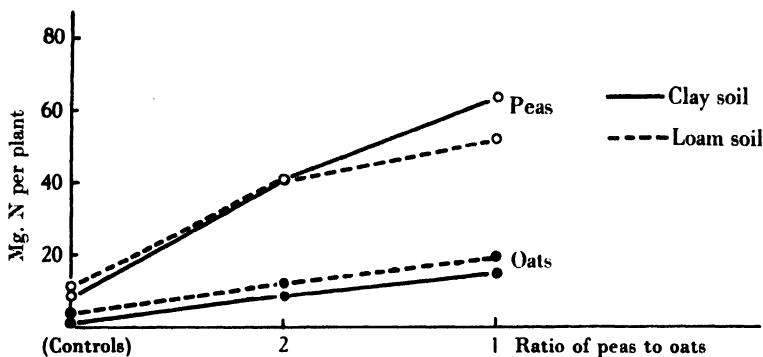
	Inoculated										Controls					
	8 to 16		8 to 16		12 to 12		12 to 12		12 to 12		8 to 16		12 to 12		8 to 16	
	peas	wheat	peas	wheat	peas	wheat	peas	wheat	peas	wheat	peas	wheat	peas	wheat	peas	wheat
<i>Exp. 1</i>																
Dry matter (g.)																
All plants	12.50	7.42	—	—	25.55	8.69	—	—	—	—	—	—	2.63	4.35	—	—
Per plant	1.56	0.46	—	—	2.13	0.72	—	—	—	—	—	—	0.22	0.36	—	—
N (mg.)																
All plants*	327.1	134.2	—	—	802.1	164.0	—	—	—	—	—	—	95.6	81.1	—	—
	(263.4)	(26.1)	—	—	(706.5)	(83.0)	—	—	—	—	—	—	—	—	—	—
Per plant	40.9	8.4	—	—	66.8	13.7	—	—	—	—	—	—	8.0	6.8	—	—
Wheat-N/peas-N (%)†	10		—		12		—		—		—		—		—	
<i>Exp. 2</i>																
Dry matter (g.)																
All plants	17.20	9.48	11.08	9.31	29.22	16.56	33.69	12.91	2.86	4.80	2.86	4.80	4.69	4.51	—	—
Per plant	2.15	0.59	1.38	0.58	2.44	1.38	2.81	1.08	0.36	0.30	0.36	0.30	0.39	0.38	—	—
N (mg.)																
All plants*	451.4	180.3	302.8	160.1	858.2	276.4	937.4	214.5	96.5	84.8	96.5	84.8	107.0	75.3	—	—
	(354.9)	(95.5)	(206.3)	(75.3)	(751.2)	(201.1)	(830.4)	(139.2)	—	—	—	—	—	—	—	—
Per plant	56.4	11.3	37.9	10.0	71.5	23.0	78.1	17.9	12.1	5.3	12.1	5.3	9.0	6.3	—	—
Wheat-N/Peas-N (%)†	27		36		27		17		—	—	—	—	—	—	—	—

\* The figures in brackets were obtained after subtraction of the controls.

† Refers to the transferred N.

each harvest the roots were removed from the soil. The two last crops were already very poor, obviously owing to lack of nitrogen. The soils thus obtained were filled into earthenware pots (9 in. diam.), whereupon the pots were autoclaved at 120° C. for 8 hr. to destroy any nodule organisms present. The associated culture experiments were carried out in the same manner as the ordinary pot experiments in quartz sand, also as regards the N-free watering solution. There was no nodulation of the peas in the uninoculated cultures.

Text-fig. 5 presents the average nitrogen results graphically.



Text-fig. 5. (See Table XVII.)

A distinct excretion of nitrogen has thus occurred also in associated cultures of peas and oats, or wheat, in clay and sandy loam soils. Since, with such soils, nitrogen determinations would not have given any reliable picture of the possible increase of nitrogen in the soil, these determinations were omitted. The excretion was therefore illustrated solely by the increase of the amount of nitrogen in oats and wheat over the corresponding controls. For the same season, the total extent of excretion could not be accurately determined, so that the only useful measure for the excretion in this case was the nitrogen transferred to the non-legume, as a percentage of the nitrogen transferred to the peas.

The results show that in clay soil the wheat received but little nitrogen (10–12 per cent) from the pea nodules, while in sandy loam soil it took up considerably more (up to 36 per cent of the nitrogen transferred to the pea). With oats the difference was less distinct, due to the fact that there were great variations between the results of the parallel experiments in sandy loam soil. However, there was some indication that the oats likewise received more nitrogen from the peas in sandy loam soil than in clay soil. In the best case, the transfer of

Table XIX

*Associated cultures of peas and potatoes in soil*

*Exp. 1. Clay soil.* 5 kg. dry soil in each pot. Peas: Torsdag; potato: Rosafolia. Peas inoculated with strain H X. The weights of the tubers employed in the different cultures were as equal as possible. The average N content per tuber was 150 mg. Period of growth: 27 June-31 August 1935. The yields include the entire plants (also roots and tubers).

*Exp. 2. Sandy loam soil.* 7 kg. dry soil in each pot. Otherwise as in Exp. 1.

	Inoculated										Controls	
	Exp. 1										6	1 to 1
	4	to	1	4	to	1	6	to	1	6		
	peas	potato	peas	potato	peas	potato	peas	potato	peas	potato	peas	potato
Dry matter (g.)												
All plants	4.54	49.38†	5.33	46.23§	6.13	55.04	7.22	75.05¶	4.13	18.49		
Per plant	1.14	49.38	1.33	46.23	1.02	55.04	1.20	75.05	0.69	18.49		
N (mg.)	113.5	592.0	215.0	502.5	157.4	733.4	198.3	913.7	114.1	297.2		
	(37.4)	(394.8)	(138.9)	(205.3)	(43.3)	(436.2)	(84.2)	(616.5)				
Per plant	28.4	592.0	53.8	502.5	26.2	733.4	33.1	913.7	19.0	297.2		
Potato-N/pea-N (%)†	788		148		1007		732					
Dry matter (g.)												
All plants**	7.60	73.76††	5.59	55.28††	12.35	72.58§§	24.19	85.92	3.59	31.4		
Per plant	1.90	73.76	1.40	55.28	2.06	72.58	4.03	85.92	0.60	31.4		
N (mg.)	251.9	724.7	153.5	550.1	380.2	735.3	828.4	974.6	63.5	377.9		
	(209.6)	(346.8)	(111.2)	(172.2)	(316.7)	(357.4)	(704.9)	(596.5)				
All plants*	62.9	724.7	38.4	550.1	63.4	735.3	138.1	974.6	10.6	377.9		
Per plant	165		155		113		78					
Potato-N/pea-N (%)†												

\* The figures in brackets were obtained after subtraction of the controls.

† Refers to transferred N.

‡ Tops 15.82 g. (264.2 mg. N), tubers and roots 32.56 g. (327.8 mg. N).

§ Tops 21.20 g. (265.3 mg. N), tubers and roots 25.03 g. (237.3 mg. N).

|| Tops 14.09 g. (207.1 mg. N), tubers and roots 40.95 g. (526.2 mg. N).

\*\* Tops 23.00 g. (345.7 mg. N), tubers and roots 52.05 g. (568.0 mg. N).

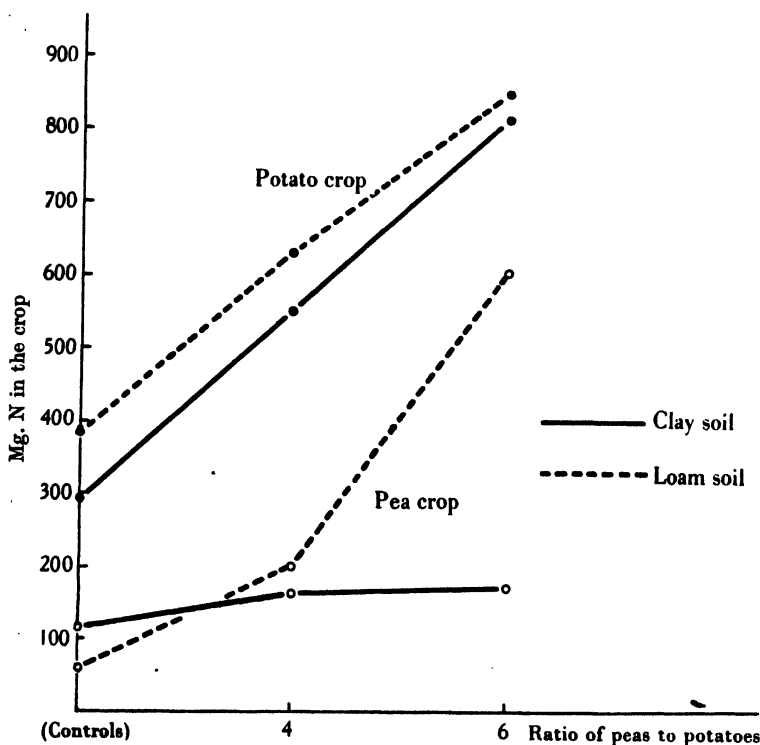
†† Tops 19.68 g. (247.5 mg. N), tubers and roots 54.08 g. (477.2 mg. N).

‡‡ Tops 14.39 g. (202.0 mg. N), tubers and roots 40.89 g. (348.1 mg. N).

§§ Tops 21.62 g. (292.6 mg. N), tubers and roots 50.96 g. (442.7 mg. N).

¶¶ Tops 26.60 g. (415.8 mg. N), tubers and roots 59.32 g. (558.8 mg. N).

nitrogen to oats in clay soil was 39 per cent, and in sandy loam soil 67 per cent, of the nitrogen transferred to the peas. The results in clay soil tended to be much more even. It should be noted that the peas grew about equally well in both kinds of soils.



Text-fig. 6. (See Table XIX.)

In associated cultures of peas and potato in clay soil the transfer of nitrogen to the potato reached a surprisingly high figure.<sup>6</sup> In the extreme case the potato received from the pea nodules ten times as much nitrogen as the pea itself. This amount is almost three times greater than that observed in quartz sand cultures where the transfer to the potato was 3.5 times greater than the uptake by the peas. The poor growth of the peas in the clay soil cultures is a clear proof of the fact that the potato has deprived the peas of their nitrogenous food. Under such conditions the nodule bacteria are more or less parasites that feed upon their host plants without a corresponding benefit to the latter.

In sandy loam soil the total amount of nitrogen transferred to the

potato is approximately equal to that in clay soil. However, in the former soil the peas themselves received so much nitrogen from the nodules that the transfer to the potato amounts only to 78–155 per cent of the nitrogen taken by the peas. Whereas the wheat received proportionally much more nitrogen in sandy soil than in clay soil, the potato received most nitrogen in clay soil. It is very difficult to explain these differences.

The average results for nitrogen *per pot* are summarized graphically in Text-fig. 6.

#### DISCUSSION

During recent years we have carried out numerous growth experiments on cultures of inoculated peas associated with oats, wheat, barley and potatoes respectively, in different proportions, using the sterile culture technique developed in this laboratory. All our experiments have shown that the non-legumes receive their nitrogenous food from the legume nodules when grown under sterile conditions, with an effective exclusion of any "foreign" bacterial decomposition of the excreted amino-acids. In most cases the nitrogen transferred to the non-legumes has been less than 50 per cent of the excreted nitrogen. In associated cultures of peas and barley with more than one barley plant in association with every pea plant, however, the barley has utilized considerably over 50 per cent of the excreted nitrogen. This could be explained by assuming that, when barley grows on a mixture of amino-acid, it also takes up some of the aspartic acid-N present<sup>1</sup>—our sterile experiments on the utilizability of various amino-acids by plants having been carried out with the individual amino-acids alone, and not with mixtures. Another explanation is that the ratio of aspartic acid to amino-acid precipitable by phosphotungstic acid varies to some extent in the different cultures. True, in each of the three pea cultures we have analysed so far, the aspartic acid-N at the start of flowering has been about 50 per cent of the total excreted nitrogen (variations 45·7–51 per cent), but this might have been accidental. The question will undoubtedly be settled as soon as more experimental data become available. It should be remembered that it is not possible to determine the ratio of the amino-acids in associated cultures wherein the non-legume utilizes part of the excreted compounds. Anyway, it is important to note that in cultures of peas and barley the percentage content of aspartic acid (total sand-N basis) is considerably higher (60–65 per cent) than what has been found in cultures of peas alone, showing that the barleys have used mainly the amino-acid precipitable by phosphotungstic acid.

<sup>1</sup> See the note on p. 589.

The extent of excretion is usually higher in associated cultures than in pure legume cultures, and rises with increasing ratio of non-legumes to legumes. When the legume is inoculated with an efficient and well-excreting strain, the amounts of nitrogen present in the sand and transferred to the non-legumes are often altogether higher than those taken up by the legume. With increasing ratio of non-legumes to peas, the growth of the peas often suffers from lack of nitrogen, caused by an increasing excretion from the nodules. A very clear example of this will be found in Table IV, Exp. 2, where the N-content of the two peas only exceeded that of the controls by 8.3 mg., while the four barley plants took up twice that quantity (16.7 mg.), a further 24.2 mg. being found as an increase of the N-content of the sand. In this case, the extent of excretion was 83.1 per cent. This result is convincing, since under the sterile conditions maintained in the experiment nitrogen can be introduced into the sand only through the activity of the nodules. It is thus seen that under certain conditions the nodules may deprive their host plant of carbon compounds which—after the incorporation of the amino-group—are given off into the medium instead of to the host plant. The factors that determine this process are still unknown. The fact remains that the nodules of a poorly grown pea may have carried out just as powerful a fixation as the nodules of a vigorously grown plant, the only difference being that in the former case the pea itself has received very little of the fixed nitrogen but has instead been compelled to give off large amounts of organic matter which has subsequently been excreted into the medium and thereby lost to the host plant. On the other hand, the excretion of nitrogenous compounds is undoubtedly a normal process which takes place also in legumes showing a maximum growth, when the excreted N is often higher than with poorly grown legumes. We have previously pointed out that the excretion obviously first removes from the nodules the excess nitrogen which the plant cannot utilize. This view is supported by our determinations showing that, when the C : N ratio in the pea is, say, 16 : 1, the same ratio in the excreted products is 3.5 : 1, so that the excretion of carbon is proportionally much less than what would correspond to the C : N ratio in the plant. Still, it is difficult to explain why the excretion may rise to such an extent that the growth of the pea is thereby greatly impaired.

The results of associated growth experiments obtained with ordinary pot cultures are, on the whole, similar to those obtained under sterile conditions. Pot experiments will naturally not give an equally reliable picture of the extent of excretion as the sterile experiments, due to the

possible interference of contaminating micro-organisms. However, the distinct parallelism of results obtained with the different experimental systems is a proof for the usability of the data from the pot culture experiments. The retardation of the growth of the pea with increasing ratio of non-legumes to peas is also clearly illustrated by the pot experiments. The fact that this phenomenon was not discernible in the experiment with uninoculated cultures growing on nitrate nitrogen is best explained by assuming that the excretion is the more extensive the more non-legumes are taking up the nitrogen from the legume nodules. It is also possible that a reduced content of oxygen in the medium, or a corresponding increase in the carbon dioxide content, might contribute to this process.

The ability of the non-legumes to deprive the peas of their nitrogenous food is strikingly illustrated by associated cultures of peas and potatoes. In quartz sand cultures with four peas and one potato plant, the potato received from the nodules 3.5 times as much nitrogen as did the peas themselves. When eight peas were grown together with one potato, the latter received only about the same quantity of nitrogen as did the peas. In the former case each pea plant received 34.7 mg. N (potato 471.8 mg.), and in the latter case 74.2 mg. (potato 575.7 mg.), so that in the former case the peas were indeed clearly deprived of nitrogen by the potato.

Our experiments with a low-nitrogen clay soil furnish a striking example of the ability of the potato to take up nitrogen from the pea nodules. In extreme cases the potato took up ten times as much nitrogen as the peas did. We wish to point out, however, that these experiments are less conclusive than those made with quartz sand cultures, since soil contains large amounts of insoluble N-compounds which might have been rendered utilizable by the plants through the action of the micro-flora in the rhizosphere of the inoculated legumes. It is not known whether such decomposition is more likely to occur in inoculated cultures than in the uninoculated controls. Nevertheless, this possibility should not be overlooked. The fact that the peas grew very poorly in the pea-potato cultures in clay soil, as compared with the corresponding experiments with peas and oats (when the oats received, in the extreme case, only 39 per cent of the nitrogen transferred to the peas), indicates clearly that the potato had depressed the growth of the pea. In sandy loam soil the transfer of nitrogen to the potato was, even in the extreme case, only about 1.5 times that to the peas. Consequently, the growth of the peas was not affected to the same extent as in clay soil. It is difficult to account for this difference, since oats, and particularly wheat plants, received

more nitrogen from the peas in sandy loam soil than in clay soil. In the quartz sand experiments, where the N-content of the medium does not interfere, the results are much more clear-cut. Still, the associated culture experiments with clay and sandy loam soils furnish unmistakable proof of the ability of the non-legumes to take up their nitrogenous food through the intermediary of the pea. They are also in perfect accord with the quartz sand experiments in showing that the non-legumes, particularly the potato, are able to deprive the pea of its nitrogen to such an extent as to depress its growth seriously.

### SUMMARY

The associated growth of peas and different non-legumes (barley, oats, wheat, and potato) was investigated both under sterile conditions and in ordinary pot cultures. In the former case the medium consisted of quartz sand, and in the latter of clay or sandy loam soil, poor in soluble nitrogen. It is shown that nitrogen was transferred from the pea nodules to the non-legumes.

In sterile cultures, where the excreted amino-acids cannot undergo decomposition, oats, wheat and barley utilized only a part of the excreted nitrogen, generally less than 50 per cent. In some instances, however, the barley took up considerably over 50 per cent of the excreted nitrogen. There was an apparent accumulation of aspartic acid in the medium of the associated cultures. In pot cultures, where the amino-acids may have been broken down by contaminating micro-organisms, the transfer of the excreted nitrogen to the potato exceeded 90 per cent.

All experiments show that with increasing ratio of non-legumes to legumes the growth of the peas suffers, obviously from lack of nitrogen. Under these conditions, the excretion from the nodules increases to such an extent that the peas receive only a small fraction of the nitrogen fixed by the nodules. The major part of the carbohydrate material furnished by the pea for the amino-acid synthesis in the nodules is excreted into the medium. This naturally causes disturbances in the growth of the pea.

Nitrate cultures of uninoculated peas associated with non-legumes show no regular changes in the growth of either type of plant, with varying ratios of legumes to non-legumes.

The maximum extent of excretion in sterile cultures was observed in an experiment with peas and barley, when 83 per cent of the total fixed nitrogen was excreted into the exterior surroundings of the nodules.



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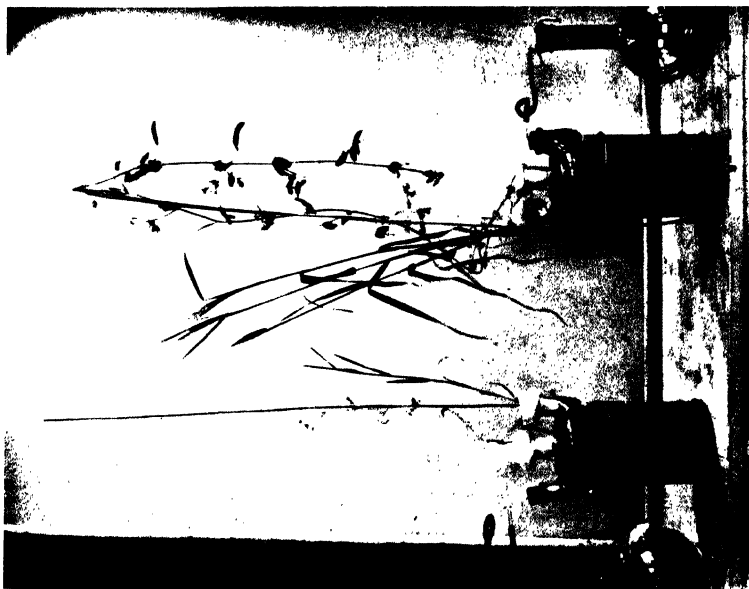


Fig. 1. Associated cultures of pea and barley in quartz sand (sterile system). Left: uninoculated. Right: inoculated (note the 3-tillered barley: for convenience in photographing the pea was bent down).



Fig. 2. Associated cultures of peas and potatoes in N-free quartz sand. I, Uninoculated; 4 peas + 1 potato; II, Inoculated; 8 peas + 1 potato; III, Inoculated; 4 peas + 1 potato.



# FERTILITY, MORTALITY, AND GROWTH RATE IN PIGS

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(With Three Graphs)

## INTRODUCTION

THE cost of producing a bacon pig is governed by a variety of factors, most of which are capable of human control. It is influenced by the amount of food consumed per lb. live weight gain, by the unit cost of food, by the number of pigs reared per litter, by the frequency with which the sow farrows, by the size and lay-out of the producing unit, by the efficiency of the labour organization, by the breed of pig, by the management of the herd, and by the business ability of the producer. In their turn these main factors are each affected by groups of subsidiary factors. The rate of meal consumption, for example, will be conditioned by the balance of the rations, by the method in which they are fed, or by the amount of waste incurred in their feeding. In the same way the effect of housing on growth rate depends on whether the accommodation is dry or damp, draughty or draught-free, well or ill ventilated, or provided with sufficient trough space. But even if these differences could be eliminated there would still be variations in production rates between individual pigs and groups of pigs due to differences in the genetic composition of the stock employed. These variations would arise between breeds and between strains in the same breed, and it is with them that the present investigation is mainly concerned.

A study of this nature is complicated by the fact that variations in feeding and management occurring between herds may mask or distort breed differences. Ideally, each breed should be kept under controlled conditions, but such a course is clearly impossible in commercial practice. In consequence, it is necessary to deal with large samples of pigs selected at random, or with smaller samples drawn from farms following approximately similar methods of management. In the latter case the value of the analysis will depend largely on the investigator's knowledge of the conditions of management governing the individual herds which compose the sample.

### MATERIAL

The material used in the following analysis was obtained during the years 1927-31 in the course of the operation of the East Anglian Pig Recording Scheme. Some of the data collected were for various reasons incomplete or covered an inadequate number of observations, and certain of the following analyses are open to criticism on these grounds. In other cases, moreover, it has been impossible to disentangle completely the various factors affecting certain relationships. The investigation can claim, however, to draw certain broad distinctions between breeds, but its main value probably lies in showing how, given sufficient data, breed characteristics may ultimately be distinguished.

### PRE-WEANING DATA

The life of the bacon pig falls naturally into two stages—the pre-weaning or suckling period when it feeds mainly on the mother's milk, and the post-weaning period, when, removed from the sow, nourishment is obtained mainly from cereals. Unlike most other forms of livestock the sow is kept solely for the reproduction of her species; she has no by-products to offer for human consumption. The whole cost of her annual upkeep must be borne by the pigs she produces, and she must be fed and cared for whether she produces pigs or not. The number of pigs reared per litter, and the frequency with which the sow farrows has, therefore, an important influence on pig production costs. The larger the number of pigs over which the cost of the sow's maintenance can be spread the lower the cost of each individual, and the greater the profit per sow. It is essential, in consequence, that breeding stock should be selected for their capacity to produce large and healthy litters.

### NUMBERS BORN AND REARED

Prolificacy is measured by the number of pigs born alive per sow, and less directly by the number of pigs reared. The former measure gives the surest indication of the fertility of the breed, as the number of pigs at weaning is probably influenced to a greater extent by external factors of management than by the capacity for survival inherited from the sow. Table I compares these factors in certain breeds and crosses found in the Eastern Counties. The data are based on nearly 2000 litters produced on about 20 farms.

The Large White and the Large White/Large Black cross pigs are drawn from approximately 12 herds. The Middle White and the Large

White/Middle White cross pigs were obtained mainly from two farms using similar systems of management, while the sows on the farm producing cross-bred pigs were originally purchased from the pure Middle White stock.

Table I. *Litter size and percentage survival 'at 6 weeks*

Breed	No. of litters	No. of pigs born alive per litter	No. of pigs alive at 6 weeks per litter	Percentage survival at 6 weeks
Large White	530	10.2	7.8	76.2
Large White/Large Black	628	9.3	7.7	83.1
Large White/Saddleback	177	9.8	7.7	79.1
Large White/cross-bred	171	10.1	8.3	82.0
Middle White	171	9.7	8.1	83.5
Middle White/Large Black	82	9.6	8.0	82.9
Large White/Middle White	86	10.4	9.0	86.2
Large Black	132	8.6	7.1	82.4

Comparing the Large White and Large White/Large Black pigs, which are the breeds most commonly used in the Eastern Counties, it will be seen that the pure-bred Large White produce roughly one pig more at birth than the Large White/Large Black cross. On the other hand, while 83 per cent of the cross-bred pigs were alive at 6 weeks, only 76.2 per cent of the Large Whites survived at that time. These figures support the opinion, commonly held in the area, that the Large Black sow is a "better mother" than the Large White. In practice, however, this quality seems to be largely discounted by the fact that the breed is less prolific. Both the Middle White and the Large White/Middle White cross pigs produced large litters, but in each case the sample under observation was small. Farrowing results for 1935, from the books of the National Pig Breeders' Association (1), are given in Table II for comparison with Table I.

Table II. *Litter size and percentage survival at weaning*

Breed	No. of litters recorded	No. born	No. weaned	Survival rate at weaning %
Large White	13,071	10.41	8.10	77.8
Middle White	1,150	9.60	7.58	79.0
Berkshire	210	8.77	6.95	79.3
Tamworth	68	7.74	6.53	84.4
Wessex Saddleback	1,094	10.11	8.32	82.3

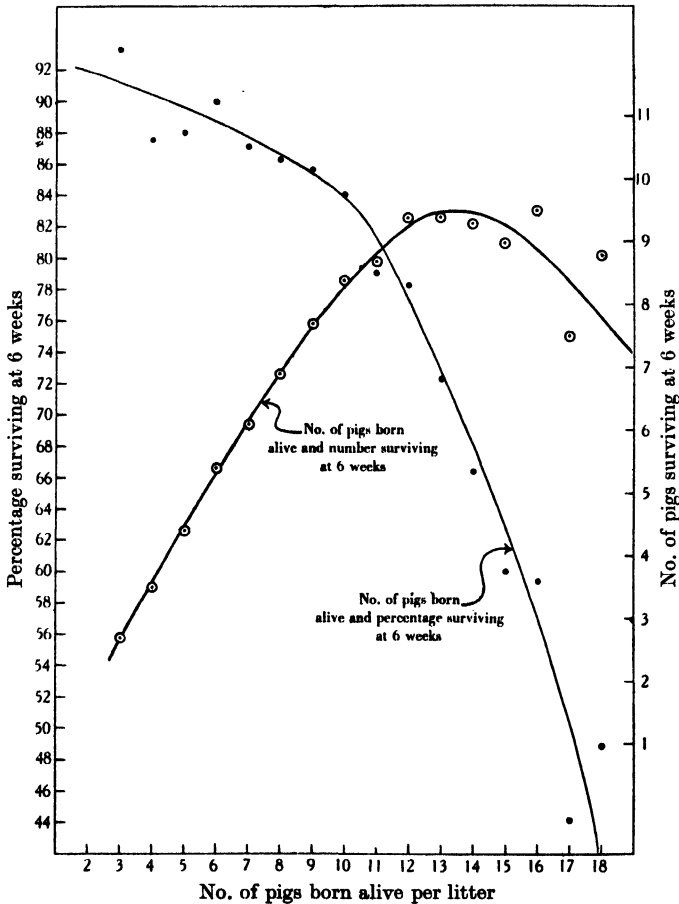
In the case of the Large White the survival rate agrees fairly closely with that in Table I; of pure Wessex Saddleback 82.3 per cent survived at weaning compared with 77.8 per cent in the Large White. Unfortunately, the Association had no figures relating to cross-bred pigs.

If the frequency distribution of litter size is examined by breed several interesting facts emerge. In the Large White group, litter size at birth ranges from 3 to 18 pigs per litter. In a total of 519 litters of this breed 8.7 per cent contained 15 pigs or over. This compares with only 1.8 per cent in 591 Large White/Large Black farrows, while in other breeds and cross-breeds the proportion of farrows with more than 15 pigs at birth was insignificant. The modal litter size in every breed is 10 except the Large White/Saddleback and Middle White, where there are 11 pigs in the modal group. Under existing conditions of management, however, there appears to be little advantage in obtaining very large litters at birth, as this is largely discounted by high pre-weaning mortality. When 1750 litters were arranged according to the number of pigs at birth it was found that an increase in pigs surviving at 6 weeks occurred up to litters of 12. In litters containing more than 12 pigs at birth the advantage of the increase in the number of pigs born was discounted by a higher death-rate between birth and weaning, and in litters of 15 or over, between 40 and 50 per cent of pigs born died before they were 6 weeks old. This fact is clearly illustrated in Graph A which shows the connexion between increasing litter size at birth and mortality up to 6 weeks.

Carmichael & Rice<sup>(2)</sup> and Murray<sup>(3)</sup> have shown that birth weight per pig declines as the litter size increases. Hammond<sup>(4)</sup> working on rabbits found the same tendency, and also that the mortality increased with the increase in litter size. Wenck<sup>(5)</sup> and Haines<sup>(6)</sup> also indicated that mortality is higher among the lighter pigs. Therefore, as individual birth weight appears to decline with increase in litter size at birth there might be a decided disadvantage in producing numerically large litters if this initial difference in weight were to be reflected in weight at 6 weeks. On examination, however, no significant difference in weight at 6 weeks was obtained between farrows in which 13, 14, 15, and 18 pigs were born. It appears, therefore, that while the average weight at birth of pigs from large litters may be less than those from small litters, the difference has disappeared before the pigs are 6 weeks old. In an investigation by Olofsson Larsson<sup>(7)</sup> in which weekly weighings were taken from birth to weaning it was found that the difference in initial birth weights had disappeared at 5 weeks.

It is probable, however, that as a result of their lower birth weight, pigs from large litters are less robust and less active during the first few days of life, and therefore more liable to be killed by the sow at this period. Moreover, as the number of pigs the sow can suckle is limited, the weaker pigs tend to be crowded out and finally to die. While they

are alive these pigs consume milk which would otherwise be available for the rest of the litter, and in so doing subject the sow to unnecessary strain. It is therefore probably advisable to kill them off at birth and to retain only as many pigs as the sow can comfortably suckle.



Graph A. Effect of litter size at birth on mortality up to 6 weeks.  
(Based on 1752 litters.)

Available evidence however suggests that while the use of reasonably prolific sows is essential to economic pig production, the reduction of pre-weaning mortality by the introduction of improved methods of management and housing, presents a more urgent problem than the increase of litter size at birth.



## EFFECT OF SEASON ON SIZE AND WEIGHT OF LITTER

Season, or month of farrow, also appears to exercise considerable influence on pre-weaning mortality. It will be observed, in Table III, that, February litters excepted, there was little monthly variation in the number of pigs born alive. Considerable variation, however, occurred in the number of pigs which survived at 6 weeks. August litters, for example, were slightly more than one pig larger than litters farrowed during November and December, and during the 6 months April to September litters contained approximately three-quarters of a pig more than those farrowed during the remaining 6 months of the year.

Table III. *Month of farrow, survival rate, and weight at 6 weeks*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Pigs born alive per litter	9.6	10.4	9.9	9.7	9.7	9.5	9.9	10.0	9.8	9.3	9.3	9.3	9.7
Pigs alive at 6 weeks	7.5	7.6	7.8	8.0	8.2	8.0	8.1	8.5	8.2	7.4	7.4	7.4	7.9
Survival rate (%)	77.6	72.7	79.2	82.2	84.3	84.5	82.3	85.0	84.3	79.9	80.0	79.8	80.8
Average wt. per pig at 6 weeks	19.1	20.2	20.9	20.3	20.9	19.3	20.0	20.0	19.5	19.8	19.9	18.8	19.9
No. of litters	247	170	152	113	167	147	212	197	147	123	143	158	1976

The quarterly results in Large White, Large White/Large Black, and Middle White litters are given in Table IV, while the monthly results for the same breeds, together with those for the pure-bred Large Black are presented in Graph B.

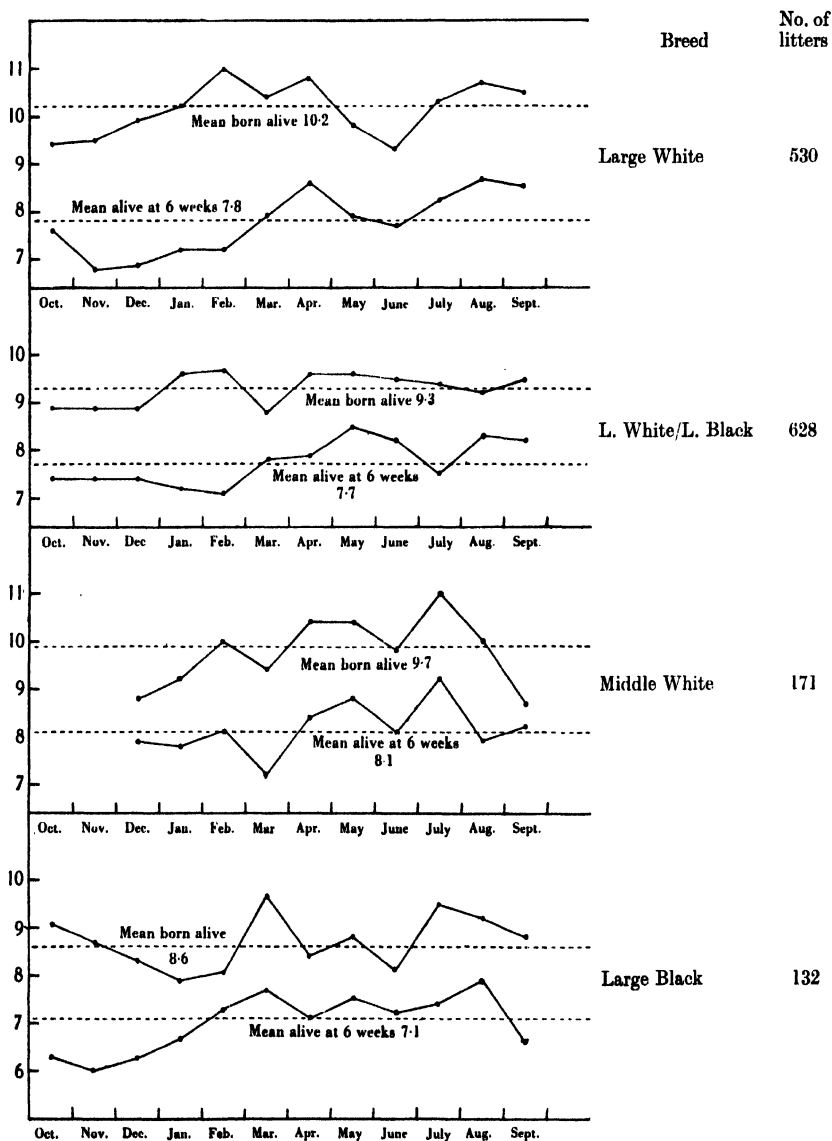
Table IV. *Season of farrow, survival rate, and breed*

Breed	Jan.-Mar.		Apr.-June		July-Sept.		Oct.-Dec.		No. of litters
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	
Large White	7.4	70.5	8.0	81.2	8.5	80.4	7.0	73.5	530
Middle White	7.7	82.3	8.4	82.4	8.7	83.6	8.3	91.9	171
Large White/Large Black	7.3	78.1	8.2	85.5	8.0	85.1	7.4	83.3	628

(1) Pigs alive at 6 weeks.

(2) Pigs alive at 6 weeks as a percentage of pigs born.

It is probable that the seasonal difference in litter size at 6 weeks is largely the result of inadequate housing, and that it could be considerably reduced by improved methods of management. At the moment, however, it appears that, assuming two litters are taken from each sow annually, roughly 10 per cent more pigs would be produced in the summer than in the winter months. In practice the difference becomes even more pronounced owing to the fact that certain producers tend to avoid winter farrows. The monthly distribution of 964 litters



Graph B. Influence of month of farrow on rate of survival at 6 weeks in various breeds and cross breeds.

## 618 *Fertility, Mortality, and Growth Rate in Pigs*

farrowed in the recorded year 1929-30 is given below, where it will be seen that just over 50 per cent of the farrows occurred in the 5 months May to September.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
No. of farrows	67	83	68	42		96	103	99	88	73	81	65	964
Total farrows (%)	6.9	8.6	7.1	4.3	10.3	10.0	10.7	10.3	9.1	7.6	8.4	6.7	100.0

In Table V litters have been arranged according to farrow number, i.e. whether they were first, second, third, or fourth litters, in order to ascertain the influence of the age of the sow on the size and weight of the litter reared.

Table V. *Influence of farrow number on size and weight of the litter*

Farrow No.	Pigs born alive per litter	Six weeks after birth				No. of litters
		Living pigs per litter	Death- rate %	Average wt.		
				Per litter lb.	Per pig lb.	
1	8.6	7.2	16.2	137.1	19.1	431
2	9.8	8.2	16.3	169.3	20.6	205
3	10.1	8.2	18.8	168.6	20.6	174
4	10.5	8.3	21.1	172.4	20.8	155
5	10.5	8.4	19.6	170.6	20.2	114
6	10.9	8.6	21.0	172.5	20.0	81
7	11.4	8.7	24.0	174.5	20.2	55
8	11.2	8.9	20.8	173.4	19.5	36
9	10.4	8.4	19.8	165.5	19.8	17
10	10.4	8.5	18.4	160.6	19.0	11
11-15	8.8	7.2	18.2	159.3	22.1	20
Average	10.1	8.2	19.1	165.6	20.3	Total 1299

From the above figures it is apparent that gilt litters are both smaller and lighter than those of mature sows; they also exhibit a lower mortality rate. Litter size appears to increase gradually up to the eighth litter, but neither fertility nor litter size is seriously impaired up to the tenth litter. This corroborates Keith (8) who, working on American breed and farrow records, including 935 litters, found that the number of pigs in the litter increased with the age of the sow up to  $4\frac{1}{2}$  years, after which there was a gradual decline; a similar result was found by Sinclair & Syrotuck (9). Moreover, up to the tenth litter there is no significant difference in the weight at 6 weeks. The percentage death-rate, however, tends to increase with the farrow number, probably due to the fact that as sows grow older they become heavier and more likely to crush their pigs.

The effect of weight variation between the individual pigs of a litter at 6 weeks on subsequent mortality is shown in Table VI where it will

be seen that the death-rate tends to be greatest in litters with a large variation.

Table VI. *Litter variation and post-weaning mortality (682 litters)*

Mean variation in litter weight at 6 weeks (%)	6-10	11-15	16-20	21-25	26-30	31-35
No. of pigs alive at 6 weeks	7.80	8.41	8.04	8.12	7.50	6.00
No. of pigs marketed per litter	7.50	7.98	7.73	7.75	6.86	5.50
Mortality (%)	3.69	5.19	3.84	4.59	9.05	8.33
No. of litters	212	243	136	59	28	4

#### INFLUENCES AFFECTING THE WEIGHT OF THE PIGS DURING THE SUCKLING PERIOD

When the pigs were grouped according to the number surviving a 6 weeks as in Table VII it was found that while the total litter weight increased with the increase in the number of pigs, the weight per pig decreased, a difference of 24.4 per cent in individual weight occurring between litters of 4 and 12 pigs. In a similar investigation in New Zealand (10) covering 399 litters it was found that the individual weight was not influenced by the number of pigs in the litter. It is possible that the apparent difference between New Zealand and English results may be due to the larger quantities of dairy products available for pig feeding in the former country. It appears that at 6 weeks Large Black

Table VII. *Effect of number of pigs in the litter at 6 weeks and individual weight at that age (943 litters)*

No. of pigs in litter	4	5	6	7	8	9	10	11	12
Average litter wt.	87.4	102.1	126.4	142.7	158.4	173.8	184.5	204.0	210.6
Average wt. per pig	21.9	20.4	21.1	20.4	19.8	19.2	18.4	18.5	17.6
No. of litters in each group	40	80	109	127	174	171	143	75	24

Table VIII. *Influence of breed on weight at 6 weeks*

Breed	No. of litters	No. of pigs	No. of pigs weighed per litter	Average wt. per pig at 6 weeks lb.
Large White	530	4127	7.8	21.0
Large White/Large Black	628	4849	7.7	19.6
Large White/Saddleback	177	1371	7.7	18.5
Large White/cross-bred	171	1418	8.3	19.7
Middle White	171	1392	8.1	18.7
Middle White/Large Black	82	644	8.0	20.1
Large White/Middle White	86	771	9.0	17.9
Large Black	132	940	7.1	21.4

## 620 *Fertility, Mortality, and Growth Rate in Pigs*

pigs are heaviest, closely followed, however, by those of the Large White breed. In view of the general claim that the Large White/Large Black cross pig grows more rapidly than the Pure White, it was rather surprising to find that at 6 weeks the Large White pigs were nearly 8 per cent heavier than those of the Large White/Large Black cross.

### RELATION BETWEEN WEANING WEIGHT AND AGE AT SLAUGHTER

There is considerable difference of opinion as to the relation between weight at weaning and age at slaughter. Wild (11) and Wenck (5) found that pigs which were heaviest at 4 and 10 weeks of age respectively were heaviest at slaughter. Hammond (12) obtained similar results with sheep, but Schmidt *et al.* (13) found no relation between the weight at 4 weeks and weight at slaughter.

Table IX. *Influence of pre-weaning weight on the slaughter age of bacon pigs*

Weight group lb.	No. of pigs in group	Average wt. at 28 days lb.	Dead wt. at slaughter lb.	Age at slaughter days	Standard age for wt. at slaughter days	Difference between standard and slaughter age days
4- 6.9	14	6.18	149.1	303.7	196.6	- 107.1
7- 9.9	136	8.68	141.4	271.3	190.5	- 80.7
10-12.9	300	11.52	146.3	260.1	193.7	- 66.4
13-15.9	329	14.28	147.2	242.8	195.5	- 47.3
16-18.9	130	17.05	149.6	232.9	195.3	- 37.6
19-21.9	30	20.06	144.7	220.8	196.9	- 23.9

From the above figures there appears, in ordinary farm practice, to be a very definite connexion between the weight of the pig at 4 weeks and the age at which it reaches a given slaughter weight, the heavy pigs reaching bacon weight approximately 80 days earlier than light. When the actual age is compared with the standard age for weight (to eliminate differences in the weight at slaughter between groups) the connexion is still more conclusive.

The point is further emphasized by comparing the growth rate of the heavier and lighter halves of 37 litters, the pigs comprising which

Table X. *Weight and age variation at slaughter within the litter*

	No. of pigs	Average wt. at 28 days lb.	Average live-wt. at slaughter lb.	Average age at slaughter days	Standard age at slaughter days	Difference between standard and actual age days
Heavier half of litters	167	18	239	297	217	80
Lighter half of litters	167	12	236	317	215	102

were weighed individually at 4 weeks old. In Table X it will be seen that a difference of 22 days, or just over 3 weeks, occurred in the time required by the two halves of the litter to reach bacon weight. The above figures emphasize the importance of litters which will grow evenly and reach bacon weight together; in fact it is probably true that an evenly balanced litter at weaning is as important as a high litter weight, if the latter is obtained by an uneven distribution of weight within the litter. That a low-pre-weaning weight does not necessarily affect the subsequent *rate* of growth is shown in Table XI, where, although the individual weight at 6 weeks was some 20 per cent greater in the smaller litters than in the large, the daily rate of increase in live weight was actually slightly higher in the litters which were lightest at 6 weeks.

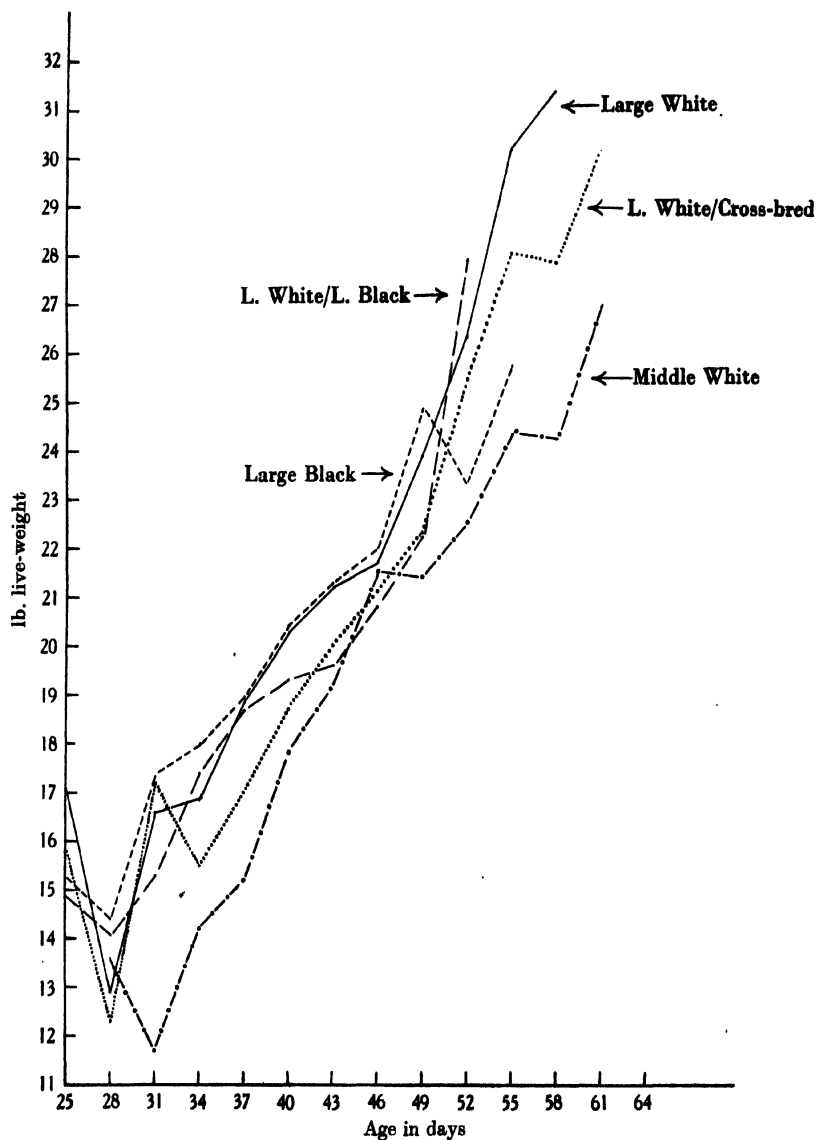
Table XI. *Influence of weight at six weeks on subsequent growth rate*

Litter size group at birth	No. of litters	Average no. of pigs weighed per litter at 6 weeks	Average wt. per pig at 6 weeks lb.	Live-wt. at slaughter lb.	Age at slaughter days	Gain per day, 6 weeks to slaughter lb.
2-5	34	3.74	24.0	184.4	206.4	0.975
6-9	161	6.85	22.2	171.3	194.7	0.975
10 and over	225	8.92	19.7	167.4	192.3	0.983

Broadly speaking, it appears that whether or not the post-weaning growth rate is affected by the weight at 6 weeks will depend on the reason for the low weight at weaning. If low weaning or pre-weaning weight is the result of disease or some inherent weakness in the pig, the subsequent growth rate may be retarded. On the other hand, if low pre-weaning weight is merely due to insufficient food, then, provided that on weaning the pigs are given adequate and well-balanced rations, they should subsequently grow as fast as heavier pigs. They will, however, take longer to reach bacon weight because as they were lighter at weaning they have more weight to put on.

#### GROWTH RATE

It was previously shown that of the breeds under discussion the Large Black produced the heaviest pigs at 6 weeks. That this superiority in weight is consistent from birth to 6 weeks is indicated in Graph C which gives the pre-weaning growth curves of the various breeds. The most interesting feature of the graph, however, is the consistent decline in rate of increase or actual drop in weight which appears to occur in every breed between the third and fourth week of life.



Graph C. Pre-weaning growth curves in various breeds and cross-breeds.

Breed	No. of pigs
Large White	4043
L. White/L. Black	4486
Middle White	1388
Large Black	938
L. White/Cross-bred	2312

It is generally recognized that the third or fourth week is a critical period in the life of the suckling pig. Olofsson and Larsson (7) have shown that about this time the milk yield of the sow falls sharply, and that unless additional food is provided a loss in live-weight, or at least in the rate of live-weight gain, is likely to occur. Moreover, the change over from liquid to solid food, particularly if the solid food is unsuitable or is obtained from dirty troughs, is often in itself sufficient to upset the digestive processes of the young pig and to lead to loss of weight. Anaemic scour resulting from the exhaustion of the iron reserves in the liver may also become apparent at this age.

The action of these various factors may actually lead to a slight loss in weight during the period. It was unfortunate that weights at a lower age were not available in order to determine the point at which this apparent decline began. Nevertheless, the figures as they stand indicate the need for careful treatment of the litter during the change over from liquid to solid food; particularly as the future performance of the pig may be largely conditioned by its treatment at this period.

#### GROWTH RATE AND MONTH OF FARROW

Consecutive litters from 77 sows were examined in order to determine whether growth rate was influenced by season of farrow. Of these sows, 48 farrowed in the January to March quarter, and again in July to September, while 29 farrowed in April to June, and October to December. The figures showed that the January to March litters reached 200 lb. live-weight 10 days earlier than litters from the same sows farrowed in July to September. In the other group of sows, April to June litters reached bacon weight a fortnight earlier than those farrowed in October to December, i.e. litters which were fattened during the warm months grew more rapidly than those fattened during cold.

Table XII. *Growth rate and grade*

	Age at slaughter days	Dead cold wt. at slaughter lb.	Grading			No. of pigs
			P. %	M. %	S. %	
Large White/Large Black	227.3	159.0	7.0	47.3	45.7	315
Large White (total)	272.3	157.4	55.1	33.1	11.8	575
Large White (Herd 21)	311.8	155.8	66.0	32.4	1.6	306
Large White (Herd 58)	205.3	156.4	73.3	22.7	4.0	101

The belief that cross-bred pigs grow more rapidly than those of a pure breed is in part at least responsible for the popularity of the Large White/Large Black cross, while the better grading of the Large White pigs is often considered to result from a slower growth rate. Superficially



this appears to be borne out by the figures in Table XII, where it will be seen that while Large White pigs required 272 days to reach bacon weight, Large White/Large Black cross pigs required only 227 days. At the same time, while 55 per cent of pigs of the Large White breed were graded as prime, only 7 per cent of cross-bred carcasses were so graded. But the number of pigs concerned in this comparison is small, and on closer analysis of the figures it is doubtful if either contention can be supported. When, for example, the rate of growth of Large White pigs in two herds where the systems of management are known is examined, it appears that in one case the pigs required an average of 312 days to reach bacon weight compared with an average of 205 in the other. Further, on comparing grading returns in these herds it will be seen that the pigs which reached bacon weight in 205 days actually gave a higher percentage of prime carcasses than those which required 312 days to reach the same weight—75 per cent compared with 66 per cent. Consequently, while it may be argued that a slow rate of growth produces a good quality carcass, it cannot be held that rapid growth must of necessity be accompanied by low grading. It is, indeed, probable that differences in growth rate and grading are greater, or at least equally great, between strains of the same breed than between the breeds themselves. It may, in fact, be possible to improve the grade of an inferior type of pig by retarding its growth or restricting its feed at certain stages of its life. In the final issue, however, the pig which brings the greatest profit is that which gives the best grade and the quickest growth rate for the minimum consumption of food. Quick growth and good grading do not appear to be incompatible, and the problem lies in the selection for bacon production of pigs of the best breed and type, and in their correct feeding and management during suckling and fattening. In this connexion it is interesting to note that of the two groups of pigs compared the growth rate in the group which took longest to fatten was retarded by a store period after weaning, while in the other group the pigs were fed to give maximum production throughout the period.

#### SUMMARY

1. Large White sows are more prolific than Large Black sows, but mortality up to 6 weeks is higher in the former breed. At 6 weeks, litters from both breeds contain practically the same number of pigs. At that age, however, pure-bred Large White pigs appear to be slightly heavier than those of the Large White/Large Black cross.
2. Sows show no significant deterioration in litter size or in litter

weight at 6 weeks up to the tenth litter, but percentage death-rate tends to increase with farrow number.

3. There appears to be little advantage in producing litters with more than 12 pigs at birth. In litters containing more than 12 pigs the addition in number is more than off-set by an increase in death-rate.

4. There is no significant difference at 6 weeks between the average weights of pigs from litters of different size *at birth*. There is, however, a difference in average weight according to the number of pigs surviving at 6 weeks; the larger the *number* the lighter the pigs.

5. The season in which the sow farrows appears to exercise a considerable influence on survival rate at 6 weeks; approximately one pig more per litter surviving in the summer than in the winter months.

6. There is a definite negative correlation between the weight of the pigs at 6 weeks and the age at which they reach bacon weight. But the *rate* of post-weaning growth of the heavy pig is not necessarily greater than that of the light pig.

7. Post-weaning mortality appears to increase with increase in litter variation.

8. Litters which are fattened during winter months take longer to reach bacon weight than those fattened during summer.

9. Rapid growth rate and high grading results are not incompatible when the conditions of suitable rations, good stock, and proper management are observed.

10. The third and fourth week of age appears to be a critical stage in the life of the suckling pig, and especial care must be exercised in its treatment at that period.

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# INVESTIGATIONS ON THE ROOT-NODULE BACTERIA OF LEGUMINOUS PLANTS

## XXI. THE GROWTH OF THE ROOT-NODULE ORGANISMS AND INOCULATED PEAS AT LOW TEMPERATURES

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THE behaviour of the root-nodule organisms of leguminous plants towards temperature was fairly exhaustively dealt with by the earliest workers on the subject(1). Numerous later investigators(2), who have subsequently determined the limits of the temperature curve (0 and 47° C.) for the growth of the *Rhizobia* on laboratory media, as well as its optimum range of growth (20–28° C.), have, on the whole, substantially confirmed Beijerinck's earlier findings. Work has also been done to determine the lower limiting temperatures for the viability of these bacteria(3,8). It has been ascertained that the cold resistance of the nodule bacteria is very high. Wilson(10), however, has observed that the total number of clover- and pea-nodule organisms in cultivated soils decreases during winter, at least in so far as the methods available for such determinations are to be relied upon. In other respects it seems that the soil temperature influences more clearly the extent of nodulation than the size of the legume crop(5).

Under Finnish climatic conditions the leguminous plants and their symbiotic organisms are scarcely likely to suffer from an excessively high temperature. Even the presumed optimum temperature for nitrogen fixation can be reached during only a short period of the growing season, the temperature of which is mostly under 20° C. It is apparent, therefore, that the chief economic importance must be attached to the effect of *low temperatures* on the *Rhizobia* and their host plants. First of all, a more comprehensive knowledge of this effect might shed some light on the advisability of inoculating leguminous crops and on the value of certain procedures for improving the temperature conditions in the soil. Such knowledge would also help us to find the most advantageous place for the legumes in the rotation. Moreover, consideration of the low-temperature relations of the leguminous crops should include the question, whether any extra nitrogen, i.e. nitrogenous fertilizers, should be applied to the legumes especially during the cold spring spells.

Under favourable temperature conditions, a nitrogenous fertilization of leguminous crops is clearly not advisable, provided that due care is taken to inoculate the roots sufficiently early with the appropriate nodule organisms and to maintain other favourable growth factors. This has been definitely proved by numerous workers. In view of the fact that detailed reviews of the literature on the N-fertilization of leguminous crops have already been made by Fred *et al.* (2) and later by Thornton & Nicol (7), it is not necessary to discuss this question here at greater length. One of the most extensive investigations in this field is Giöbel's work (4) on the effect of inorganic nitrogen upon the function of the nodules and the fixation of nitrogen. However, no definite attempt seems to have been made earlier to ascertain whether the restricted N-fixation of legumes, growing at lower temperatures, could be profitably compensated by an early application to the soil of nitrates or ammonium salts.

The purpose of the pure-culture experiments recorded here was to compare the ability of some strains of clover- and pea-nodule bacteria to grow at lower temperatures. The pot-culture experiment with peas was made to gain some preliminary information of the possible effect of nitrogenous fertilization on the growth and nitrogen fixation of peas, growing at a relatively low temperature.

## EXPERIMENTAL

### I. *The growth of some strains of Rhizobium at lower temperatures*

In order to elaborate a practical and reliable method for comparing the growth rate of pure cultures at different temperatures, it was necessary to make some preliminary experiments concerning the composition of the medium and the estimation of the amounts of bacterial substance produced during the experiment. As a result of these trials the following nutrient medium was chosen for use:

K <sub>2</sub> HPO <sub>4</sub> ...	... 1.00 g.	Yeast extract <sup>1</sup> ...	100 ml.
MgSO <sub>4</sub> .7H <sub>2</sub> O ...	... 0.20 g.	Clover extract <sup>2</sup> ...	100 ml.
CaCO <sub>3</sub> ...	... 10.00 g.	Gelatine ...	120 g.
Asparagine ...	... 2.00 g.	Water ...	... to 1000 ml.
Saccharose ...	... 10.00 g.		

<sup>1</sup> The yeast extract was prepared by autoclaving an aqueous suspension (1:20) of commercial baker's yeast for 1 hour at 120° C., the suspension being afterwards centrifuged.

<sup>2</sup> The clover extract was obtained by boiling fresh clover plants in an equal weight of water. The liquid was squeezed off from the mass and filtered or centrifuged. After sterilization in the autoclave the extract could be kept in stock for several months without losing its growth-promoting effect on the root-nodule bacteria.

## 628    *The Root-Nodule Bacteria of Leguminous Plants*

For the solidification of the medium, gelatine was found to be better than agar. A drawback in the use of agar cultures is especially the difficulty of removing the bacterial growth from the surfaces, due to the cracking of the medium and its shrinkage from the walls of the flask. Comparative experiments with the same basal solution gave the following values for the bacterial matter obtained from gelatine and agar cultures (Table I).

Table I. *Growth of pure culture of Rh. trifolii (strain "A 6 III") in 7 days on gelatine and agar media at 19–20° C.*

Flask No.	Gelatine (14%)			Agar (1.5%)		
	Dry matter mg.	N mg.	N %	Dry matter mg.	N mg.	N %
1	575	51.0	—	193	16.9	—
2	581	49.6	—	164	14.1	—
3	573	49.4	—	170	15.3	—
4	535	47.3	—	167	15.3	—
5	520	43.0	—	179	16.1	—
Mean	557	48.1	8.64	175	15.5	—

It is seen that the bacterial crop obtained from agar cultures was only about one-third of the crop obtained from gelatine cultures. This difference is probably ascribable to the lower nitrogen content of the agar medium. On the other hand, the nitrogen percentages in the bacterial crops were similar, being about 9 per cent of the dry matter. As the gum produced by the *Rhizobium* makes it difficult to count the bacterial cells, it was necessary to use other methods than counting, for the estimation of the amount of growth in the pure cultures. In all experiments described here the cultivation and harvesting of the bacterial mass was accomplished in the following way.

The cultivation of the bacteria was carried out in fairly large flat culture vessels: a type of Roux flask. Each of these vessels had a capacity of about 1.2 l., and the area of the growth surface, when 250 ml. of gelatine were used, was about 300 sq. cm. For the inoculation of the cultures, the bacterial growth from two gelatine slants was suspended in 100 ml. of sterile water, 5 ml. of this suspension being used for the inoculation of each culture flask. The cotton-plugged vessels were then kept 11–14 days at a constant temperature. At the end of the experiment, all flasks were kept for about an hour in the refrigerator at 4–7° C. About 50 ml. of cold water was then added to the flask, and the slimy bacterial growth was cautiously removed from the gelatine or agar surface with a platinum wire triangle and poured into a 250 ml. centrifuge cup. The remaining bacterial growth was then washed off with three 50 ml. portions of water. If necessary, glass beads were used for loosening the slime. The mucous mixture in the cup was heated to 50° C., acidified with a few drops of

HCl, stirred and centrifuged. The clear liquid was poured off, stirred with water and centrifuged again several times. In the first experiments the bacterial mass thus obtained was dried at 100° C. and weighed.

As the amount of growth in the different cultures was satisfactorily illustrated by the values for the nitrogen content of the bacterial crops, these values were later used as the sole basis for measuring the growth at the different temperatures. This obviates the necessity of a careful removal of the acid by washing which often causes difficulties in the separation of the bacterial growth on centrifuging.

Table II. *Growth of pure cultures of Rh. trifolii and Rh. leguminosarum at different temperatures in 13 days. 250 ml. of gelatine medium in each culture*

Temp. ° C.	<i>Rh. trifolii</i> "A 6 III"			<i>Rh. leguminosarum</i> "H 10 I"		
	Dry matter mg.	Total N in bacterial crop		Dry matter mg.	Total N in bacterial crop	
		mg.	Relative values		mg.	Relative values
5	112	9.2		110	8.7	
5	106	8.8	5 (5)	105	8.5	4 (5)
5	80	6.6		85	6.9	
10	540	45.8		727	60.2	
10	409	44.4	24 (24)	704	58.9	32 (34)
10	556	37.9		649	55.2	
19-21	2173	186.9		2063	179.5	
19-21	2112	181.7	100 (100)	1977	170.0	95 (100)
19-21	1897	173.7		1801	167.3	

The first temperature experiment (Table II) was made in order to study the growth of two strains of nodule bacteria at temperatures of 5, 10 and 20° C. Both strains were of Finnish origin. The medium in all cultures consisted of 250 ml. of the gelatine medium described on p. 627. The results show that the methods employed for the cultivation of the bacteria and estimation of the crops gave satisfactorily concordant values in the parallel cultures. On the basis of the nitrogen yields it can be concluded that the growth of these two strains at 5° C. was about 5 per cent, and at 10° C. about 32 per cent, of the best growth noted in this experiment at 20° C. It is admitted that the relative values thus obtained do not illustrate, in the best manner possible, the retardation of growth of the different strains at lower temperatures. The nitrogen yields of each strain should rather be compared with the result obtained with this strain at its optimum temperature. However, as the use of gelatine media in these experiments made it impossible to study the growth of the bacteria at their probable temperature optima, the relative values have been calculated in percentages of the maximum yield in

## 630 *The Root-Nodule Bacteria of Leguminous Plants*

each series. For want of a more reliable basis for comparison, the use of both of these relative values will undoubtedly facilitate an examination of the results.

In the following experiment (Table III) the growth of three strains of clover-nodule bacteria was studied at temperatures of 6, 11 and 19–20° C. These strains were originally isolated from localities of different climatic conditions. The strain of most “southerly” origin was isolated in this Institute from a soil sample from Egypt. The Swedish strain was kindly supplied by Prof. Barthel, Stockholm, while the Finnish strain “A6 III” was isolated from a soil sample supplied by the Leteensuu Experimental Station. The Egyptian strain failed altogether to grow at

Table III. *Amounts of nitrogen in the bacterial crops of three strains of Rh. trifolii from different countries. Incubated 13 days at different temperatures. 250 ml. of gelatine medium in each culture*

Temp. ° C.	“T.a.” Egypt			“T.r.” Sweden			“A6 III” Finland		
	N mg.	Relative values		N mg.	Relative values		N mg.	Relative values	
6	0.7	0	(0)	1.5	1	(1)	19.8	10	(12)
6	0.3			0.8			15.5		
11	11.4	6	(6)	23.5	12	(14)	71.1	38	(46)
11	10.6			19.9			64.4		
19–20	181.3			157.9			148.2		
19–20	178.2	100	(100)	—	88	(100)	143.8	81	(100)

6° C., and even at 11° C. its growth on the surface of the medium was scarcely visible to the naked eye. The northern European strains, especially the Finnish one, produced appreciably higher nitrogen yields at lower temperatures. At room temperature, on the other hand, the Egyptian strain showed distinctly the best growth.

The same bacterial strains were also included in the following experiment (Table IV). In addition, another Swedish strain, and a Finnish strain “A3 I” were included. It was known from previous plant-culture work that the nitrogen-fixing power of this latter Finnish strain was exceptionally low. Two of the temperatures in this experiment, namely, 7 and 13° C., were slightly higher than before. The quantity of medium in each culture was 150 ml., against 250 ml. in all other experiments, and this fact should be duly considered in examining the results. A comparison of the nitrogen yields and the relative values shows that the growth of the different strains at the lower temperatures was similar to that in the earlier experiment. The Finnish strain which causes a very weak nitrogen fixation in inoculated clover showed growth quite comparable with that of the efficient nitrogen-fixers at each temperature.

Table IV. *Amounts of nitrogen in the bacterial crops of five strains of Rh. trifolii from different countries. Incubated 14 days at different temperatures. 150 ml. of gelatine medium in each culture*

Temp. ° C.	"T.a." Egypt		"T.p." Sweden		"T.r." Sweden		"A6 III" Finland		"A3 I" Finland	
	N	Relative	N	Relative	N	Relative	N	Relative	N	Relative
	mg.	values	mg.	values	mg.	values	mg.	values	mg.	values
7	0	0 (0)	3.8	2 (3)	10.0	6 (6)	24.6	15 (15)	25.4	16 (17)
13	40.3		66.3		71.0		73.5		83.9	
13	34.5	23 (24)	64.9	41 (48)	68.3	43 (43)	73.3	45 (45)	80.1	51 (54)
19-20	165.0		136.1		164.1		166.9		154.5	
19-20	154.6	99 (100)	—	84 (100)	158.5	100 (100)	156.0	100 (100)	151.2	95 (100)

Table V. *Amounts of nitrogen in the bacterial crops of four strains of Rh. leguminosarum from different countries. Incubated 11 days at different temperatures. 250 ml. of gelatine medium in each culture*

Temp. ° C.	"Užice" Yugo-Slavia		"P.P." Sweden		"H 10 I" Finland		"H 7 I" Finland	
	N	Relative	N	Relative	N	Relative	N	Relative
	mg.	values	mg.	values	mg.	values	mg.	values
7	5.3		6.8		15.0		16.0	
7	3.9	2 (4)	5.2	3 (4)	13.2	7 (7)	15.3	8 (9)
13	27.7		54.5		91.6		64.7	
13	26.6	14 (22)	48.0	27 (35)	83.7	46 (46)	62.4	33 (38)
19-21	125.6		147.8		202.0		168.8	
19-21	121.4	64 (100)	142.8	76 (100)	182.1	100 (100)	165.1	87 (100)

For a comparison of the growth rates of *pea*-nodule bacteria (Table V) from localities of different climatic conditions, a Yugoslavian strain "Užice" and a strain "P.P." from Sweden were available. An efficient nitrogen-fixer "H 10 I", and a weak fixer, "H 7 I", were also included. At the lower temperatures, the Yugoslavian strain, like the Egyptian, showed a poorer growth than the northern strains, especially the Finnish ones.

The above experiments with pure cultures of nine different strains of nodule organisms have clearly shown that the different strains are not similar in their ability to grow at lower temperatures. Those pure cultures which had been isolated from Finnish soils produced distinctly more new cell matter at 6-13° C. than did parallel cultures of Swedish strains. Still more clear-cut were the differences between the Finnish strains and the corresponding strains from southern Europe or North Africa.

The ability of the nodule organisms to grow at low temperatures may have also practical significance. If the lowering of temperature retards symbiotic nitrogen fixation by the different strains, to different extents, then the development of the legumes may depend largely on the ability



## 632 *The Root-Nodule Bacteria of Leguminous Plants*

of their nodule bacteria to fix nitrogen at low temperatures. Hence the size of the legume crop in a cold season may depend on whether the strain used as inoculum is able to act at low temperatures, exactly as it often depends on its nitrogen-fixing power in general (9).

### II. *Effect of an early nitrogenous fertilization on the nitrogen nutrition of peas at low temperatures*

It must be pointed out, however, that the question of the degree of nitrogen fixation by nodule bacteria at low temperatures cannot be definitely settled merely by examining the growth of pure cultures at different temperatures. As the nitrogen fixation by these organisms apparently takes place only in the nodules of the living legume, plant experiments are needed to throw light on the influence of temperature. From a practical viewpoint, the whole problem can be summarized thus: Does a reduction of temperature more greatly affect the assimilatory rate of the host plant, or the rate of nitrogen fixation by its symbiotic nodule organism? Which of these is the limiting factor?

Should the lower temperature affect the activities of the host plant less than it affects the functions of the bacteria, then the host plant will suffer from lack of nitrogen, which can be compensated by nitrogenous fertilization. If, again, the function of the bacteria, even in the cold, keeps pace with the carbohydrate metabolism of the host plant, nitrogenous fertilization would not be expected to help any more than it does at a more favourable temperature. Conversely, the behaviour of an inoculated legume, growing at a relatively low temperature, towards nitrogenous fertilization should be an indication of the extent to which the strain used for inoculation has been able, under these conditions, to satisfy the nitrogen requirements of its host plant.

Simultaneously with the pure-culture experiments recorded above, a series of pot experiments with peas was put up in an unheated greenhouse. The purpose of this pot experiment was to make a preliminary study of the effect of nitrogenous fertilization on the development of legumes, inoculated with certain strains of nodule bacteria, and growing at a relatively low temperature. The inoculated seeds (Torstai pea) were sown on 15 March 1936 in thirty earthenware pots, each containing 2.3 kg. of dry, pure quartz sand. The pots were watered with a basal nutrient solution of the following composition (g. per l.):

$\text{KH}_2\text{PO}_4$	...	0.20	KCl	...	...	0.10
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	...	0.05	$\text{FeCl}_3$	...	...	Trace

10 g. of chalk was mixed with the sand in each pot in order to maintain a suitable reaction. 2 l. of the above nutrient solution were applied, in small portions, to each pot during the period of growth. Further watering was done with tap water, to bring each pot to a constant weight. Eight seeds were initially sown in each pot, but only five seedlings were allowed to develop. The temperature of the greenhouse during germination, was 15–18° C. At the end of the germinating period (8 days from sowing), the temperature gradually fell. From 27 March onwards the room was kept as cool as possible by continuous ventilation, care being taken, of course, that the temperature of the room did not fall below zero.

The arrangement of the experiment (cf. Table VI) was the following. The seeds in all the pots were inoculated with pure cultures of pea-nodule bacteria, fifteen pots with strain "P.P." (imported from Sweden) and another fifteen pots with the Finnish strain "H10 I". Five pots in both groups were left without any nitrogenous fertilization throughout the experimental period, while another five pots were supplied, on 27 March, with 150 mg. N in the form of  $\text{Ca}(\text{NO}_3)_2$ , dissolved in the watering solution, and the remaining five pots in each group with the same amount of nitrogen as  $(\text{NH}_4)_2\text{SO}_4$ .

Table VI. *Plan and principal results (averages of five parallel pots, five plants in each) of a nitrogen fertilization experiment with peas, grown in a cold greenhouse. Sown 15 March, harvested 15 June 1936*

No.	Inoculated with strain	Added N (27 March)		Mean dry wt. of one plant g.*	Mean length of stem cm.	Total N per plant			Protein N per plant mg.*	Amino N per plant mg.*
		Nutrient	Per plant mg. N			Tops mg.	Roots mg.	Tops and roots mg.		
1	"P.P."	—	—	4.91	129	115.2	19.6	134.8	73.1	56.3
2	"	$\text{Ca}(\text{NO}_3)_2$	30	5.23	131	123.0	19.4	142.4	69.7	52.4
3	"	$(\text{NH}_4)_2\text{SO}_4$	30	4.41	132	117.6	19.8	137.4	69.6	51.8
4	"H10 I"	—	—	4.69	131	117.2	22.8	140.0	75.8	57.4
5	"	$\text{Ca}(\text{NO}_3)_2$	30	4.79	128	116.7	22.5	139.2	73.1	54.7
6	"	$(\text{NH}_4)_2\text{SO}_4$	30	4.37	128	114.4	21.8	136.2	72.1	53.5

\* Without roots.

In order to obtain reliable data of the temperature conditions during the experiment, the temperature of certain pots was continuously measured with a thermoelectric apparatus. Copper-constantan thermocouples were sunk into the sand in eight of the pots. The passive junctions of the couples were kept in a glass paraffin-oil tube which was placed into a thermos flask filled with crushed ice (temperature 0° C.). The ends of the series of thermocouples were connected to a galvanometer which

## 634 *The Root-Nodule Bacteria of Leguminous Plants*

drew a continuous temperature curve on a paper scale, run over a drum operated by clock-work. Direct control measurements were made twice a day. The mean sand temperatures for each day, as well as the maximum and minimum values, could then be calculated from the curves. The mean temperature of the pots for the growth period from 27 March onwards was 11.8° C.

It is thus seen that the plants grew throughout at a somewhat lower temperature than usual, especially during the earlier part of the growth period. This circumstance apparently retarded the development of the plants to such an extent that they had only just passed the flowering stage when harvested, after 92 days from sowing, although the usual growing period of the Torstai pea, in field trials carried out in southern Finland, is reported to be about 84 days. The low temperature did not, however, cause any visible disturbances in the plants, which, from the beginning, developed soundly and with a normal colour. The only discernible differences between the different treatments were that the growth of the peas in the parallel pots which were not supplied with mineral nitrogen, was more even than that of the N-fertilized plants, and that in the  $(\text{NH}_4)_2\text{SO}_4$ -group the lower leaves of the plants began to wither earlier than in the other groups. Flowering commenced practically simultaneously in all groups, at about 77–79 days after sowing. At the time of harvesting, the leaves and stems of all the plants were still mostly green.

The results in Tables VI and VII were obtained from the harvest made on 15 June, in the following manner. In each pot, the tops were cut, measured and dried at 50–60° C. as one sample. After weighing, the entire sample was finely ground, and total nitrogen was determined from a weighed portion by the usual Kjeldahl method. The roots were likewise harvested separately for each pot, washed under the tap, dried, weighed and analysed for total N. From the finely ground samples of tops, bulked samples were made for each different treatment. Three such samples of 2 g. each were then taken for: (I) the estimation of total nitrogen, (II) successive determinations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , soluble organic N, and insoluble organic or "protein" N, and (III) amino-N determination according to van Slyke. For comparison, these determinations were also made on numerous original, unmixed samples. The determinations in (II) were carried out as follows:

2 g. of the sample was weighed into a 100 ml. Erlenmeyer flask and 50 ml. water was added, after which the flask was kept in a boiling water-bath for 30 min. After cooling, 25 ml. of 10 per cent trichloroacetic acid solution was added, the volume was

made up to 100 ml. with water, and the flask was allowed to stand overnight. The mixture was then filtered and the residue washed with 2.5 per cent trichloroacetic acid. The total nitrogen in the residue ("protein" N in the tables) was determined by the Kjeldahl method. The filtrate was neutralized to about pH 5 with NaOH, made alkaline with MgO, and the ammonia was distilled into  $N/10$   $H_2SO_4$ , which was then made alkaline with NaOH and re-distilled into  $N/10$   $H_2SO_4$  for titration. The residue after the first distillation was treated with the Devarda-reagent and NaOH, and distilled twice for the determination of nitrate N. The soluble organic N was then determined from the residue by the official Kjeldahl method. Amino N was estimated according to van Slyke(6), except that the hydrolysis of proteins was accomplished by autoclaving in 5 per cent HCl at 15 atm. for 4 hours.

An examination of the results thus obtained shows that:

(1) An early supply of nitrate nitrogen slightly increased (6.5 per cent) the *dry-matter* yield of tops of peas inoculated with the Swedish strain "P.P.", while with the Finnish strain "H10 I" the effect of nitrate was not marked. Fertilization with ammonium sulphate slightly lowered the dry matter yields of tops (by 7-10 per cent).

(2) Artificial nitrogen supply caused a slight increase in the *total nitrogen* of plants inoculated with "P.P.", while in conjunction with "H10 I" it brought about an insignificant decrease in the nitrogen yields. With all treatments, the total N content of the roots of the plants inoculated with "H10 I" was 10-16 per cent higher than in the "P.P." group. The better ability of the Finnish strain to grow at lower temperatures has probably resulted in a more abundant nodule formation. A difference in the extent of nodulation was in fact noted at harvesting.

(3) Artificial N fertilization has to some extent unfavourably affected the *protein synthesis* by the plants (Table VII). Table VI shows the yields of protein N and amino N, calculated on the basis of these determinations. It will be seen that *the yields obtained exclusively by biological N fixation are the best*. The high nitrate content of the plants

Table VII. *Fractionate analysis of the nitrogenous constituents of the dried tops of pea plants from the pot experiment recorded in Table VI. N fractions as percentage of total N*

No.*	NH <sub>4</sub> N %	NO <sub>3</sub> N %	Soluble organic N %	Protein N %	Amino N %
1	2.73	1.04	27.44	63.42	48.88
2	2.62	5.78	30.98	56.66	42.58
3	2.50	1.09	33.01	59.18	44.06
4	2.39	0.97	28.14	64.67	48.94
5	2.95	3.15	28.03	62.62	46.87
6	3.02	1.16	29.21	63.00	46.74

\* Nos. 1-6 as in Table VI.

## 636 *The Root-Nodule Bacteria of Leguminous Plants*

supplied with nitrate nitrogen appreciably reduced the value of the crop, as judged by the standards of nutritional physiology.

It may be mentioned that there were considerable variations in the nitrate content of different parallel samples in the nitrate group. In the plants inoculated with the Swedish strain nitrate formed from 1.98 to 9.42 per cent, and in plants provided with the Finnish strain, from 2.65 to 8.82 per cent, of the total nitrogen. It was also observed that nodulation was invariably very poor in those pots which produced crops notably high in nitrate. Nevertheless, the deviations between the total N yields from the different parallel pots were relatively small.

### SUMMARY

The present paper records some investigations on the growth of the clover- and pea-nodule organisms (*Rhizobium trifolii* Dangeard and *Rh. leguminosarum* Frank), and the nitrogen fixation of the pea, at low temperatures. Growth of the bacteria was studied with pure culture experiments in large flasks, in which the bacteria were grown on a solid gelatine medium. The amount of nitrogen in the bacterial mass thus obtained was used as the basis of comparison. The fixation of nitrogen by peas was studied with a pot-culture experiment in a relatively cold greenhouse. Inoculated plants were grown in quartz sand, and were supplied at an early stage with mineral nitrogen either as  $\text{Ca}(\text{NO}_3)_2$  or  $(\text{NH}_4)_2\text{SO}_4$ . The plants were harvested after flowering and analysed. The main results of these experiments were the following:

(1) Pure-culture experiments with five strains of clover-nodule bacteria and four strains of pea-nodule bacteria showed that at 6–13° C. the strains imported from more temperate climates grew distinctly less well than did the northern European strains. Even between the Swedish and Finnish strains there were clear differences: the latter formed the more bacterial matter in the cold. It may be appropriate to consider such differences when selecting cultures for inoculating leguminous crops.

(2) In the pot-culture experiment, the application of mineral nitrogen (30 mg. N per plant) to inoculated peas kept at relatively low temperatures had very little effect on the amount of dry matter produced. The slightness of this effect was similar to what has been previously observed in experiments at more favourable temperatures. Nitrogenous fertilization slightly lowered the amounts and percentages of total N present as protein and amino nitrogen, in the crop; but nitrate application somewhat increased the nitrate content of the crop. Hence, it may be concluded

that, even under these low-temperature conditions, the application of artificial nitrogen has not had a favourable effect on the size or quality of the crop.

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# THE WATER CONSUMPTION OF SUCKLING SOWS

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THE importance of an adequate water supply for all farm livestock is generally recognized, but, with the exception of milking cows, few exact determinations of the amount of water drunk by farm animals have been made. This gap in knowledge is evidenced by the fact that the recognized textbooks speak only in generalities on this question. That cows' milk yields are considerably reduced if an insufficiency of water is available is the common belief, and, by analogy, it might be expected that some of the cases of milk shortage in suckling sows should be ascribed to shortage of drinking water. Nevertheless, little, if any, attention has been paid to the water requirements of suckling sows. When, therefore, the system of tethering sows was adopted on the Cambridge University Farm, it was decided to seize the opportunity presented of determining individual water consumption.

Under the tethering system each sow is allotted a hut, in which she lives from about a fortnight before farrowing till her litter is weaned: this hut carries a covered water trough which cannot be upset and from which she alone can drink. In these circumstances it was easy to determine the daily water consumption. When a sow was first put on the tether chain her trough was filled to a definite mark, and then throughout her period on the tether the trough was filled up to the same mark twice daily, the amount of water required being recorded. The daily consumption was measured for the whole time the sow was on tether in two experimental periods: in two further periods the water consumption was recorded from the time of farrowing onwards. With sows farrowing at intervals an experimental period was necessarily prolonged, and the rule was made that recording should continue until 6 weeks after the latest farrowing in the group. Periodically the huts were moved a few yards and the troughs were filled to the mark before and after moving, so that errors did not arise through splashing in transit. The other water available to the sow during the whole period was that contained (about 5 lb. a day) in the bran mash fed for the first 3-4 days after farrowing, and such as she obtained from the grass; it must be pointed out that the latter was appreciable on some days, and therefore the figures here given for consumption should be regarded as conservative estimates.

All the animals were of the Large White breed and only three of the thirty-seven used were gilts. The grass did not provide a large proportion of their food, and in many cases they ate up to 16 lb. per head per day of appropriate cubes.

The work was divided into four experimental periods, two of these being in winter and two in summer. The mean daily consumption for the week preceding farrowing and for the first 6 weeks after farrowing are given in Table I.

Table I. *Water consumption of sows before and after farrowing*

Period and dates	Mean temp. (° F.)	No. of sows	Av. no. of pigs per litter (weaned)	Av. wt. per pig at 6 weeks (lb. )	Mean consumption per sow per day (lb.)							Mean for weeks 1-6 after farrowing
					Week preceding farrowing	Weeks after farrowing						
						1	2	3	4	5	6	
(1) Nov. 1934- Mar. 1935	44.85	10	7.6	22.6	37.2	41.7	40.6	39.5	40.3	39.5	37.4	39.8
(2) June 1935- Aug. 1935	64.58	11	9.3	24.1	38.2	43.3	47.2	45.9	45.9	45.9	46.5	45.8
(3) Dec. 1935- Mar. 1936	36.40	8	7.9	22.3	—	45.5	43.9	43.5	45.1	40.9	40.7	43.3
(4) Apr. 1936- June 1936	53.05	8	7.9	—	—	39.3	43.6	44.3	44.0	37.7	39.1	41.3
— All periods	—	37	8.2	—	—	42.5	43.9	43.3	43.8	41.3	41.2	42.7

In the first period there was little difference in the water consumption before and after farrowing, but in the second period the pre-farrowing consumption was significantly ( $P < 0.01$ ) less than the post-farrowing consumption. It will be noted that the figures for pre-farrowing consumption are very much higher than those obtained by Evvard *et al.* (4). As regards the consumption after farrowing the only consistent trend that can be observed is a fall in the fifth and sixth weeks: the figures for these last 2 weeks were significantly ( $P < 0.05$ ) lower than those for the first 4 weeks in the last period, as was the case for all periods combined. When it is considered that during the first week after farrowing the sows were receiving approximately half a gallon per head per day of water in their bran mash, the general inference is that there is a slight tendency for consumption to decrease throughout the suckling period.

In each period the variation between sows was highly significant; for all periods together the standard deviation was 15.4 lb. a day, which was 36 per cent of the general mean. Furthermore, individual sows varied considerably in their requirements from time to time, the standard deviation "within sows and weeks" (that is, when the variations between sows, and between periods of lactation, had been eliminated) being 14 per cent of the general mean. Finally, the sows varied widely



from day to day within a week for no apparent cause, the extreme variation in daily consumption being from less than 5 lb. to as much as 95 lb.

The average consumption was, approximately, 43 lb. per head per day, and there was surprisingly little difference in consumption between winter and summer periods. It was decided to explore the question of the relation between weather and water consumption more thoroughly, and for this purpose the figures for the first week after farrowing were neglected. For each sow the subsequent 34 days were divided into seventeen 2-day units, the consumption in these units being correlated with maximum and minimum temperature and rainfall; the object of using a 2-day unit was to minimize errors arising through slight variations in the times of drinking. The correlation coefficients shown in Table I were obtained "within sows and units", that is, when the variations between sows and that due to the progress of lactation were excluded. The absence of a figure for rainfall in period 2 is explained by the fact that there was a drought throughout the time of observation.

Table II. *Correlation of water consumption with climatic factors (2-day units)*

Period	No. of observations	Correlation coefficients between water consumption and		
		Max. temperature	Min. temperature	Rainfall
1	170	+0.294	+0.226	-0.033
2	187	+0.089	+0.008	—
3	136	+0.080	+0.025	-0.114
4	136	+0.148	-0.096	-0.031

With rainfall none of the correlations approached significance, and with temperature only those in period 1, both of which were significant ( $P < 0.01$ ); despite their significance, however, these two correlation coefficients were very low. The coefficients shown in Table II support the conclusion drawn from Table I that water consumption is very little influenced by weather.

It was thought probable that sows yielding much milk would drink more water than those yielding little, and therefore correlation coefficients were determined between water consumption on the one hand and number of pigs in the litter and weight of litter at 6 weeks on the other. For this purpose water consumption in weeks 2-6 after farrowing was used, and there were thirty-seven observations for the correlation with number in litter, and twenty-nine for weight of litter. The correlation coefficients in the former was +0.226, and in the latter +0.269; neither of these coefficients was significant.

## DISCUSSION

There is no need to dilate upon the many essential functions that water performs in the animal body, but some discussion of the intake and output of these sows may be attempted, though this involves the use of rough estimates. In addition to the 43 lb. actually drunk, the sows had access to grass; in the management of the herd it is generally assumed that during the summer period they would eat the equivalent of 4 lb. of concentrates in the form of grass. If this assumption is justified they obtained 12 lb. of water per day from the grass and this figure conforms closely to that given by Tod(8). It may be that the fact that the consumption was little higher in summer than in winter should be partly ascribed to the greater intake of grass in the summer. On the other hand, the water actually drunk during the summer of 1935 was less than 5 lb. per day higher than in the other periods; the summer period of 1935 was characterized by a drought and very little grass was available to the sows at that time. It must be confessed that any estimate of the water derived from the grass consumed must be almost a pure guess, but if a figure is to be put to it 10 lb. daily for the summer period, and 5 lb. for a winter period, would appear reasonable. As regards the concentrates fed, the greatest amount of water included, except for the bran mash immediately following farrowing, would not exceed 2 lb. per day. A further source of water would be the fat of which the sow's body was denuded during the suckling period; in its oxidation this fat would produce its own weight of water. It was only possible to weigh some of the sows in these experiments, but it appears that they fell in weight, roughly, from 400 to 300 lb. between farrowing and weaning; it appears, therefore, that this source would not provide more than an average of 2 lb. of water a day. It thus appears that the average total water intake was, in lb., 43 as drinking water, 5-10 from the grass, 2 from the concentrates, and 2 from the breakdown of body fat; this total is approximately 55 lb., but it must be emphasized that this figure is subject to considerable error, especially in the component provided by the grass.

No effort was made to determine the actual milk yield of these sows. Hughes & Hart(5), from a summary of the literature on the subject, concluded that the average yield of milk throughout the lactation was 6-8 lb. per day. Bonsma & Oosthuizen(1) obtained an average of 6.5 lb. for the daily yield of sows. It is, therefore, probable that some 7 lb. of water would be included daily in the milk produced. It must be admitted, however, that from the present records it is impossible to produce evidence

that water consumption was related to milk yield; it is true that in the first period the sows drank 5 lb. less before, than after, farrowing, but at the time of farrowing a loss of nearly 50 lb. in body weight would occur, which might very well account for this difference in consumption.

If 7 lb. be allowed as actually contained in the milk, 48 lb. per day remain as the amount available for maintenance purposes. Mitchell & McClure<sup>(6)</sup> state that the water requirement of chickens, sheep and swine is 1 c.c. per cal. produced, though they observed that the voluntary intake of cows is much bigger than that. According to Brody *et al.*<sup>(2)</sup> a 350 lb. sow would produce nearly 5000 cal., and that number of cubic centimetres is equal to 11 lb. It thus appears that in the case of sows, also, the voluntary intake is much above the basal requirements. Besides the water lost to the body by respiration, perspiration and in the excretion of urinary products, a considerable amount is excreted in the faeces. This latter source of loss would depend to a considerable extent on the nature of the food. Sheehy<sup>(7)</sup>, for example, maintains that substances such as bran or succulent feeds have the property of retaining water in the large intestine, and so have special value in feeding.

Actual records of the water consumption of pigs are very scarce in the literature. Evvard *et al.*<sup>(4)</sup> found that pregnant gilts only consumed, on the average, 7.5 lb. per day, a figure very much lower than those found here. Clausen<sup>(3)</sup> gave the standard consumptions for five Danish research stations as 6.5 lb. of skimmed milk, 6.4 lb. of corn and 3.2 lb. of water daily for a 200 lb. fattening pig. It would appear that the total intake of water approximated to 10 lb., but it is not surprising to find a much higher figure for a suckling sow of nearly double the weight.

#### SUMMARY

The water consumption was determined of thirty-seven Large White suckling sows that were tethered on grass. The mean consumption was 43 lb. a day, the variation between sows, and of particular sows from day to day, being particularly wide. The consumption was practically unaffected by weather and was nearly the same in summer and winter; it was impossible to demonstrate any relation between consumption and number or weight of litter, but there was evidence of a slight decrease as lactation progressed.

It is evident from a brief discussion that exact balance experiments as to the partitions of water for its various uses in the body of the pig are badly needed. It is hoped that the facts given here may draw attention to this point which has been neglected in animal nutrition studies.

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